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BASIC PSYCHOLOGICAL STUDIES OF THE EFFECTS OF INCAPACITATING AGENTS

U.S. Army Chemical Center
Contract No.: RFP-55 DA18-108-405-CML-738
Order No. CP-0-405-10875

FINAL REPORT
July 31, 1962

Contractor: Indiana University Foundation
Research Division
Bloomington, Indiana

Principal Investigator: Roger W. Russell

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Virginia Fox, B.A., conducted the pilot study described in Chapter IV, section I.

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I. INTRODUCTION

"Incapacitation" is defined in terms of changes in behavior, changes which disrupt the organization and performance of normal activities. The final criteria for testing incapacitating agents must, therefore, involve measures of behavior. There are many such measures which might be included in programs designed to screen chemical agents for incapacitating properties. Each of these measures may be affected by the characteristics of the organism under study, the conditions under which behavior is generated for test purposes, the research design employed in the test, and properties of the chemical agent administered. Obviously, effects of these confounding variables and of their interactions would influence the interpretation of results provided by any screening program and, thereby, the decisions about actions to be taken in regard to the chemical agent under study. From the practical point of view these variables contribute to the risks of labelling a new agent as worthy of development when in fact it is nor or, on the other hand, of failing to identify a new agent which is promising. The present research project arose from the wish of the U.S. Army Chemical Center to minimize these risks by obtaining as full information as possible about the effects of the confounding variables on measures of behavior which might be included in screening programs.

In accordance with specifications in the contract proposal, the first six months of the project were devoted to examining the objectives of a program for identifying and evaluating potential incapacitating agents. The objectives were summarized and requirements for achieving them specified in the project's Report No. 1 submitted in September 1960. This report listed desired characteristics of incapacitating agents, defined the concept of "incapacitation" in behavioral terms, discussed considerations in research design where chemical agent-behavior interactions are involved, and specified requirements to be met by any measures of behavior which might be used in studying such interaations.. The report also outlined a four-phase screening program two phases of which involve studies of infrahuman animals and two, human subjects; the first three phases employ laboratory tests and the fourth, field tests.

The remaining year and a half of the contract has been devoted to empirical studies. In this short period consideration could be given to only a few of the issues which deserve attention. A decision was made to concentrate on problems occurring in Phase 2 of the suggested four-phase program, which involves "screening in depth" using infrahuman animals subjects; any screening program for evaluating effects of chemical agents on behavior must begin with research using such subjects. Time and the level of effort supported by the contract made it impossible to undertake all the research required to develop a full screening program of the Phase 2 type. Instead the research has concentrated on studying a variety of behavior measures and of research designs, all of which are candidates for inclusion in a final screening program as described in the project's Report No. 1. For some of the behavior measures, comparative data have been collected on effects of three prototype chemical agents; for other measures, data could be obtained on only one or two of the agents. The number of subjects in the experiments has also been limited, in several instances to the extent that replications of the studies would be necessary before conclusions could be drawn with confidence. Despite these limitations, evaluation of the results clearly provides information relevant to the kinds of practical issues described earlier which affect the screening of chemical agents for psychoactive properties.

The general plan of the present report begins with a consideration of research designs, general procedures, and some properties of the chemical agents used, before describing in detail the experiments on individual behavior measures. Descriptions of the latter, which constitute the largest section of the report, are followed by a description of the pharmacological studies undertaken preliminary to, or concurrent with, the behavior studies. Finally, there is a consideration of the implications of the research results and a brief concluding summary.

II. RESEARCH DESIGNS AND GENERAL PROCEDURES

In his treatise, "The Design of Experiments", R.A. Fisher (1942) emphasized a point which is accepted as a truism, but which is of particular importance for any study involving chemical agent-behavior interactions:

> "If the design of an experiment is faulty, any method of interpretation which makes it out to be decisive must be faulty too. It is true that there are a great many experimental procedures which are well designed in that they <u>may</u> lead to decisive conclusions, but on other occasions <u>may</u> fail to do so..."

Research design is particularly important in research of the present kind because of the number of variables capable of influencing any set of observations. Carry-over effects illustrate one of the most troublesome results of the operation of such variables. Repeated administrations of the same chemical agent, even when interdose intervals are of considerable length, may produce a cumulation of effects. The sequential administration of two or more different chemical agents may give rise to one of three effects depending upon the pharmacological and biochemical modes of action of the particular agents involved: one may summate with the other or one may potentiate the other, in both cases full effects being produced at reduced dose levels; one may antagonize another, thus resulting in a failure of a full dose of the latter to give its full effects. When any of these effects are operating there lurks the danger of faulty interpretation of results. As the results of the present series of experiments show, carry-over effects occur frequently under conditions similar to those which characterize practical screening programs. Precautions may be taken to minimize the risk of carry-over effects by allowing sufficiently long intervals between administrations of the chemical agent under study. Other precautions may be taken by designing the research in such a way as to determine whether or not carry-over effects did in fact occur and to identify more fully the nature of the effects.

This is only one example of the kinds of variables which may affect the interpretation of results in experiments planned to determine effects of chemical agents on behavior. One of the objectives of the present project was to study empirically the operation of several different research designs under experimental conditions of this kind. The present section describes the experimental variables studied and the designs employed and discusses certain related issues of general procedure.

Experimental variables

By studying techniques for generating and measuring the various behavior patterns it was possible to control a number of variables which might otherwise have affected the research results: some were eliminated and others were held constant throughout the period during which the independent variables under study were being systematically varied. The three independent variables of primary interest were: differences in chemomorphology of the chemical agents studied, dose levels administered, and times after administration at which tests were made for possible effects of an agent on the behavior patterns measured. The use of several dose levels provides data for computing the dose-response characteristics of the agents administered; repeated measurements of behavior after administration make it possible to compute the time-response characteristics, including peak-effect times and durations of effects.

The behavior patterns constituting the main dependent variables will be described in a later section, which reports the details of the experiments on behavior. These dependent variables were all measures of performance, rather than of learning. Report No. 1 presented the view that the main military uses for incapacitating agents seem to involve the temporary deterioration or disruption of performance in skills already acquired. The acquisition of new skills is usually a fairly lengthy process involving periods of training which take place in the zone of the interior rather than in a theater of operations; to incapacitate the acquisition process in a significant way would require an incapacitating agent with very persistent after effects of acute administration or repeated administrations of a shorter-acting agent. Performance of skills already acquired would appear to be the primary target in identifying and evaluating potential agents; performances which it may be militarily desirable to alter range from simple motor skills to complex decision-making. Dependent variables studied in the present project were selected to sample this range to the extent possible under the time limitations of the research period. Standard measures of these variables provided data on level of performance under the experimental conditions and on intra- and interindividual variability in performance.

Several research designs were used in studying relations between these experimental variables.

Randomized groups design

When subjects are to be administered only one dose of an agent it is possible to use a randomized groups design in which n subjects are assigned at random to one of k treatments. If each treatment is considered to be of equal importance, it is advantageous to have equal n's in the various treatment groups. The results may be analyzed by analysis of variance which provides information about the significances of the different treatments, dose levels in the present studies, and of interindividual differences among subjects within treatment groups. The use of this type of design will be illustrated with data from studies of two behavior patterns.

Latin square design

It is a great advantage in screening chemical agents if the same subjects can be used for repeated testing. Even when the most efficient and economical techniques for measuring behavior have been designed, establishing stable baselines of pretreatment performance is time-consuming; the experimenter's "investment" in each animal is great even before an agent is administered for the first time. One way of minimizing the total number of subjects would be to test each at all treatment or dose levels. The Latin square design is one possible way of accomplishing this; variations of this design have been used in several of the experiments to be reported later.

Latin squares were formed by having the number of subjects in each square equal to the number of dose levels administered equal to the number of administrations. Randomization within each square was restricted in such a way that each dose occurred only once at each administration and each subject received each dose only once during the drug series. Wherever time permitted the Latin square was replicated using a new group of subjects.

Latin square design with replication of the same square

It is possible that the order in which doses of the chemical agent are administered may influence the effects of the agent on the behavior measured. The use of a Latin square design with replication of the same square permits the isolation of a sum of squares corresponding to the particular orders in which the agent was in fact administered. This design was used in several of the experiments to be reported later.

Other research designs

It has not been possible to work with certain other research designs which were scheduled for consideration. One, the Latin square design with balanced squares, has advantages by providing an estimate of residual effects of immediately preceding treatments as well as of treatment effects (Williams, 1949). Another makes it possible to obtain a better estimate of residual effects by adding an additional session or column to the Latin square which duplicates exactly the treatments of what otherwise would be the last column of the square; this means that each treatment is preceded equally often by each other treatment including itself (Cochran and Cox, 1957). A third is referred to as a "factorial design" in which the variables involved are studied in all possible combinations in the same experiment; although this type of design has certain advantages, it also has the disadvantage that the number treatment-combinations or the number of different subjects to which treatment-combinations must be administered may become so large as to make the experiment administratively impracticable.

Carry-over effects

A requirement in the analysis of the Latin square design is that the observations for the different doses are independent, i.e., that a behavior measure at one dose level for a particular test session is not dependent upon the effects of doses administered during an earlier drug series. When this requirement is not met, the treatments are said to have carry-over effects; under such circumstances, differences in residual effects from one drug series to another could lead to an order in the results due to treatment interaction rather than treatment *per se*. There is always danger of carry-over effects when the same subjects are given a series of different treatments.

One way of eliminating the possibility of carry-over effects is to provide sufficient time intervals between successive administrations of the chemical agent for the residual effects of one treatment to dissipate completely before the next treatment is undertaken. In studies of chemical agent-behavior interactions, recovery from residual effects may involve three separate, but potentially related, variables: changes in behavior, presence of the agent in the body, and changes in biochemical systems affected by the agent. The time characteristics of these variables may be quite different. For examples, studies of the effects of the "tranquilizer" reserpine on behavior indicate that the drug is absorbed and metabolized rapidly, reaching an asymptotic minimum within two hours and persisting without further significant change over a period of 48 hours (Plummer et al, 1957); significant behavior changes begin to appear after this asymptotic level has been reached and disappear while it still persists (Russell, 196?); brain serotonin and norepinephrine levels decrease rapidly after administration of the drug, 90 per cent or more disappearing within four hours, but do not return to normal level for about seven days (Shore and Brodie, 1957). Since any of these three classes of variables may contribute to residual effects when the same subjects are used in a series of treatments, all must be considered in deciding upon the duration of intervals between successive administrations of the chemical agent under study in any experiment. Ideally the intervals would be sufficient to encompass the duration of the slowest recovery process, but, for the economy of the screening program, the duration would be no longer than necessary.

Within the time limits of the present project it was necessary to depend upon preliminary observations of the effects of the various drug treatments for information upon which to base inter-treatment intervals. The procedure was to determine the time required for each behavioral measure affected to return to its pretreatment baseline;an additional margin of safety was added to establish the intervals between drug administrations for subsequent experiments. The frequent appearance of significant carry-over effects in the analyses of results indicates that this procedure in itself was not adequate and suggests the need to supplement information about behavioral recovery processes with analogous information of a pharmacological and biochemical nature. A beginning was made during the course of the project to obtain the relevant pharmacological data for the agents used; these data will be discussed later. To obtain corresponding information about changes in biochemical events poses the most difficult problem of all, since it requires knowledge of the modes of action of the chemical agents involved.

Without these kinds of information it is impossible to rule out the hypothesis that carry-over effects may be responsible for the order found in an experiment involving repeated drug treatments of the same subjects. The use of designs involving repeated treatments of the same subjects is appealing from the point of view of economy in research time and effort, since observations on fewer subjects are required. However, the appeal.

> "...must be evaluated against the worth of less 'iffy' designs such as (1) random assignments of m individuals to each of the T treatment (or experimental) conditions or (2) the used of matched cases with matching on the basis of some relevant variable(s) or on the basis of pretest measures of the dependent variable under consideration (McNemar, 1962)".

Dose-response relations

Application of the research designs described above provide data which may be analysed to reveal relations between various dose levels of a chemical agent administered and concommitant quantitative changes in the behavior measured. The standard procedure in analysing the data from the present series of experiments has been: first, to plot levels of performance for each dose level over the time period of an experiment; second, to calculate the significance of the dose-response relations at the time when the agent had its peak effect, using analysis of variance techniques appropriate for the particular research design involved; and, third, to compute a "median behavior dose" or BD_{50}, which represents the dose of a particular drug at its peak effect necessary to produce a significant change in 50 per cent of the sample studied. The method for computing BD_{50} will be described later, when it can be illustrated using results from present studies.

Time-response relations

Chemical agents differ in the speed with which they produce their effects after administration and in the duration of these effects. Report No. 1, in discussing the desired characteristics of incapacitating agents, pointed to two major features of their time-response relations: first, it is generally desirable that the onset of effects occur in one hour or less following administration of an agent, although an agent which had a delayed time of onset would also be of interest; second, it is important to know the duration of action once the effects have been produced, with the possibility that it may be desirable to have a family of agents with varying durations of effective action. To obtain data from which these time characteristics may be determined, the standard research design for the present experiments has included periodic measures of behavior at intervals following administration of an agent. When a measure of behavior is sensitive to a particular dose of an agent, repeated tests can be expected to show a change in baseline performance, developing after administration and returning to baseline level as time passes. In analyzing the time-response data the procedure adopted has been to plot the relations, to determine peak-effect times, and to specify durations of action in terms of time between administration of an agent and the recovery of a measure of behavior to the extent that it no longer differs significantly from its predrug baseline.

Predrug baseline performance

Several references have already been made to the very basic roles that data on the stability of predrug baseline performance plays in the analysis and interpretation of the present experimental results. Stability is determined by comparing the similarity of measurements obtained from observations of a subject's performance in a standardized screening situation on two or more occasions with periods of time intervening between observations. The procedure used in the present experiments was to measure each subject's performance during a predrug period when the behavior was being shaped by training. Practice was continued until an asymptote in level of performance was reached. Analyses of these predrug performance levels and comparisons with performance levels following recovery from drug treatments will be described later when the data for specific experiments are presented.

Subjects

During recent years many different species of infrahuman animals have been used in research on drug-behavior interactions. In some instances selection has been motivated by an interest in a particular species; in other instances, the use of several species has provided a basis for comparative studies of behavior; in still other instances animals have been used as "tools" for research, with the assumption that the results of such research may have implications for drug-behavior interactions in man. The selection of animal subjects for the behavioral screening of chemical agents is based upon this latter assumption. The validity of the assumption needs further systematic testing, although it has been empirically established for certain biological phenomena and has been put to practical application particularly in the medical sciences. Because of the importance of the assumption to the screening of chemical agents for their psychoactive properties, the question of validity was discussed in Report No. 1 and will be considered later in the context of the present experimental studies. It is obvious that the question in its general form is as applicable to other aspects of a screening program, e.g., pharmacological, toxicological, physiological, biochemical, as it is to the behavioral.

Report No. 1, in outlining a general program for screening incapacitating agents, envisaged the use of several infrahuman animal species as subjects for particular phases of the program. Within the limits of the present project it was necessary to concentrate on only two species if other objectives were to be achieved. The rat and the monkey were chosen, the former because of the detailed information already available about its behavior and the latter because of its phyletic position close to man.

All rats used as subjects were from the Holtzman Albino stock (Holtzman Company, Madison, Wisconsin). This source of supply provided vigorous, docile animals which were more homogeneous in weight than other standard sources contacted, a desirable feature for certain pharmacological determinations to be reported later. Only male animals were used and all were between three and four months old when started on their training trials. Each was housed in a separate cage during the entire duration of its participation in the project. They were fed on standard Wayne Lab Blox for Rats and Mice (Allied Mills, Chicago), *ad libitum*, except when on a limited intake schedule required to control food-hunger motivation.

Squirrel monkeys, *Saimiri sciurea*, also served as subjects in some of the experiments to be reported. All were purchased from suppliers who obtained them directly from their native habitats. Their ages were unknown, although all appeared to be relatively young animals.

Behavior measures

The view was presented earlier that the present project should be oriented toward the study of performance rather than learning. Even with this limitation, there still remained many different behavior patterns from which to select measures of performance to be included in a battery of screening tests. The bases upon which selections may be made have been described (Russell, 1960). These were considered in making choices for the present experiments. The selections discussed in Report No. 1 were used, with some modifications, all measures being likely candidates for inclusion in a final test battery. Each will be described later when specific experiments of the present series are considered.

III. CHEMICAL AGENTS

The major objective of the present project was to study the nature of variables affecting the validity of methods for screening potential incapacitating chemical agents. Therefore, the choice of agents to be used in the project was not limited by a requirement to screen new compounds of unknown value. After discussions with the first Contract Project Officer it was decided that the needs of the project and of its unclassified security status would best be met if psychoactive chemical agents already in general use, i.e., not restricted to use as incapacitating agents, were selected for study. The three agents - Librium, pentobarbital sodium, and ethanol - were chosen from a list of prototype drugs suggested by the Psychopharmacology Service Center, National Institute of Mental Health for use in basic research on behavior (September 1961 number of the PSC Bulletin).

Librium, or methaminodiazepoxide, has been described as a "psychosedative"; it is used clinically with reported success in the treatment of anxiety and tension where its most obvious characteristics are its sedative and muscle relaxant properties. Pentobarbital is one of the barbiturates with a relatively short duration of action. Ethanol, or ethyl alcohol, is also rapidly absorbed and catabolized; like pentobarbital its acute effect on the central nervous system is to act as a depressant upon the cerebral cortex. Relations between the actions of these drugs and their effects on the behavior measures studied in the present project will be discussed later in this report.

The drugs were administered orally or intraperitoneally in aqueous solutions, which were made fresh at least once daily during experimentation. To assure maximum stability and solubility of the Librium and pentobarbital solutions, no attempt was made to neutralize them. Instead, placebo solutions were prepared of dilute acid and base, which approximated the acidity and alkalinity of the more concentrated drug solutions administered (see Table 1).

Table 1

Conditions Selected for Drug Administration

Drug	Preparation	Placebo	Route	Maximum dose (per kg)	Peak Effect Time[1] (hours)
Librium hydrochloride	Aqueous soln. @ 0.1 ml/100g	.06 NHCl	Oral	32 mg.	1.00
			IP	<8 (>4)mg	0.25
Pentobarbital sodium	Aqueous soln. @ 0.1 ml/100g	.08 NNa_2CO_3	Oral	40 mg	0.50
			IP	<10 (>5)mg	0.25
Ethanol	50% aqueous solution	Water	Oral	>3.0 ml[2]	0.50
			IP	0.2 ml	0.25

[1] Peak effect time observed in gross behavioral effects, i.e., on motor coordination and startle and pain responses. Observations made only at time intervals of $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, and more hours after drug administration.

[2] 3.0 ml constituted the practical limit which could be administered to ad lib fed 450-500g. rats via oral intubation, although gross behavior measures were relatively unaffected at this level of 50% ethanol.

In order to more closely approximate the clinical situation, all drugs were administered orally for the behavior studies. Oral administration was done via a stomach intubation technique, utilizing a 1 ml tuberculin syringe with a 3 inch, 18 gauge needle modified with a slight curvature and a rounded ball of solder to blunt the tip. Preliminary studies showed no significant effect on the various behavior test performances due to the intubation procedure alone.

Significant changes in behavior may occur at any dose level between zero and the level at which toxic effects to the body appear. For purposes of the present research drug levels for the behavioral experiments were established by preliminary behavioral toxicity studies, to be described in detail later, in which the effects of various doses of each drug on selected indices of locomotor coordination, general activity, startle response and response to pain were systematically observed and rated. The minimum effective dose for these gross behavioral variables was used as a guide in setting the maximum drug level for the more quantitative experiments on behavior. The other end of the effective working range was defined by a zero dose, or placebo treatment. Doses within this range were related in decreasing fractional steps of one-half between succeeding dose levels, a selection which proved empirically to be effective for describing dose-response characteristics of the three drugs studied. The standard dose levels used in these experiments for the three drugs studied were:

Librium - 0, 4, 8, 16, 32 mg/kg,
Pentobarbital - 0, 5, 10, 20, 40 mg/kg, and
Ethanol - 0, 0.25, 0.5, 1.0, 2.0 ml/kg.

IV. EXPERIMENTS ON BEHAVIOR

A. Introduction

The preceeding sections have set the background for a detailed examination of the several experiments designed to study effects of the chemical agents upon behavior. In describing research designs, procedures and analyses of results for individual experiments, it will not be necessary to repeat in detail the general considerations which have already been discussed; instead emphasis will be placed upon special features of each experiment and upon the results obtained.

Most of the experiments to be reported were designed to provide information about a single behavior pattern; occasionally two related patterns were studied in a single experiment. Seven experiments were completed to the point of obtaining data for at least one replication of the research design. In order to study a wide range of behavior patterns, all experiments were planned to use a minimum number of subjects per replication and to add replications when desirable. Because of the limited time available it has not been possible to replicate some of the experiments sufficiently to provide final, definitive results; these experiments must be considered as pilot studies of the kind which are particularly characteristic of developmental research. In addition to detailed data concerning specific drug-behavior interactions, the experiments as a whole provide information about factors which affect the practical task of screening chemical agents for their psychoactive properties and illustrate the steps involved in developing a standardized screening battery of behavioral measures of the type discussed in Report No. 1.

B. Food intake

In research designed to study effects of chemical agents on behavior, food intake may serve as a useful measure in two principal ways. Food intake is often very sensitive to disease processes and, therefore, may be helpful in monitoring the general state of health of animal subjects involved in an experiment. For example, the most frequent diseases in our rat colonies involve respiratory disorders, which, if diagnosed early, can be treated successfully; experience has shown that one of the first symptoms of such disorders is a decrease in food intake.

Measures of consummatory responses also are one way to quantify appetitive drives (Miller and Barry, 1959), food intake serving as a measure of hunger. The effects of a drug on this consummatory response may provide useful information as to whether motivation is involved in the behavioral mode of action of the drug. In studies employing food-hunger as the means of motivating the behavior under study, e.g., the straightaway approach response in the present experiments, the amount of food intake is often controlled for each subject as a means of establishing and maintaining consistent hunger levels; drugs may affect these levels despite such controls and thereby alter behavior. More difficult to discern are effects of interactions between changes in food-hunger and another form of motivation which may be serving as the primary motivation. For example, Knopfelmacher et al (1956) report behavioral effects of thiamine and reduced caloric intake which "...suggest that some interaction occurred between the two drives, escape from water and food-hunger, even though the latter was never reinforced at the completion of any trial". By recording food intake, the possibility that this kind of interaction might have occurred during an experiment may be examined.

Records of food intake were maintained during most of the experiments of the present series in which rats were used as subjects. In the majority of instances the records were necessary parts of the procedure for controlling level of food-hunger motivation; the procedure imposed a maximum limit on food-intake and, therefore, made any measure of the latter invalid as an index of ad libitum consummatory behavior.

However, detailed records of such behavior were kept during the course of two experiments, both to be described later: one was designed to study effects of Librium on a straight-away escape response and the other, effects of pentobarbital on the same response. Analyses of these two series of data are reported in the paragraphs which follow.

Research design and procedure. The research design and procedure are discussed in detail in section "D" below. In essence, a 5 x 5 Latin square design with two replications of the same square was used in studying each drug. Each replication required five animals, five drug dose levels and five drug series, each beginning with the administration of a single dose and observations continuing for six days. Animals were assigned at random to the five rows of the design.

The 10 Holtzman albino rats who served as subjects were adult males whose body weights had reached the characteristic plateau of approximately 500g. They had not participated in any previous studies. All were housed in separate cages during the experimental period and were fed and watered ad libitum. The food was available in pellet form from metal hoppers which were attached exterior to the mesh cage walls. Daily food consumption was measured by weighing the hopper contents at the beginning and end of each 24 hour period. Spillage was not recorded, but appeared to be minimal and fairly constant.

Records of food intake were kept during the course of two experiments: one involving the administration of Librium and the other, pentobarbital. Doses for the former were 0, 4, 8, 16 and 32 mg/kg and for the latter, 0, 5, 10, 20 and 40 mg/kg; all doses were administered orally. In order to obtain data necessary to determine time-response relations between the absorption-metabolism of each drug and any changes which might occur in the consummatory response, food intake measures were recorded, in grams, at the end of each 24 hours during the six days between drug administrations.

Results. Tables 2 and 3 summarize the results of the studies in terms of mean food intake at each of the five dose levels for the two drugs administered. Data are provided for each of the six days following drug administration. Examination of the data in these tables and of plots made from them suggest that neither drug had any significant effect of a systematic nature on this consummatory response. The absolute level of food intake remained very steady throughout the six days following each drug administration. There is no obvious evidence of any concommitant variation between mean intake and drug dose level. Analyses of variance for the Librium and pentobarbital data summarized in Tables 4 and 5, substantiate these observations; in neither case is the F-ratio significant for "dose". Two significant variance terms do appear. The significant F-ratio for "weeks" in the Librium data indicates the presence of carry-over effects during the sequence of weekly drug administrations. There is no evidence for such effects in the pentobarbital results. This distinction between the two drugs will appear again in other behavior measures to be reported later. Variability among subjects was significant during the pentobarbital series, but not during the Librium tests.

Since chemical agents which increase the variability of group behavior may "incapabitate" the normal functioning of the group, the effect just referred to was analyzed further. Inter-individual variability during each test session was computed and expressed as a standard deviation, s. Standard deviations for both the Librium and pentobarbital series are given in Tables 6 and 7. It is of interest to know whether variability under drug conditions differed significantly in any instance(s) from variability under the corresponding "0" or placebo condition. Tests for such significance can be made by obtaining an unbiased estimate of the population variance for each s^2 and comparing the variance estimates for each dose level with the estimates for corresponding placebo conditions. The comparison is made by expressing each pair as a ratio, which is an F-ratio (Senders, 1958). These computations were made for the data in Tables 6 and 7; the instances in which variance under a drug condition differed significantly from variance under its corresponding placebo condition are indicated by asterisks in the tables. Only one difference in variability was found to be significant in each of the two drug series, a finding which might be attributed to chance. However,

Table 2

Mean Food Intake[1]: Librium

Time after administration (Hours)	Dose (mg/kg)				
	0	4	8	16	32
24	28	21	24	28	30
48	32	28	32	28	24
72	29	28	33	28	27
96	30	30	33	31	25
120	27	28	25	26	27
144	29	29	32	23	27

[1] In grams.

Table 3

Mean Food Intake[1]: Pentobarbital

Time after administration (Hours)	Dose (mg/kg)				
	0	5	10	20	40
24	29	34	35	34	31
48	31	37	37	40	36
72	32	39	37	38	42
96	37	31	31	33	41
120	29	34	32	31	31
144	37	34	37	29	38

[1] In grams.

Table 4

Food Intake: Librium

Summary of Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
Dose	1,816.56	4	454.14	3.21	ns
Subjects	1,162.56	4	290.64	2.05	ns
Weeks	3,925.76	4	981.44	6.93	<.01
Error	1,700.48	12	141.71		
Total	8,605.36	24			

Table 5

Food Intake: Pentobarbital

Summary of Analysis of Variance

Source of Variance	Sum of Squares	d.f.	Mean Square	F	P
Dose	420.96	4	105.24	----	ns
Subjects	3,324.56	4	831.14	7.19	<.01
Weeks	422.16	4	105.54	---	ns
Error	1,388.08	12	115.67		
Total	5,555.76	24			

Table 6

Interindividual Variability[1] of Food Intake[2]: Librium

Time after administration (Hours)	Dose (mg/kg)				
	0	4	8	16	32
24	8.78	12.12	8.89	9.43	8.31
48	7.14	10.54	8.66	10.91	6.71
72	9.22	12.37	9.85	10.86	11.14
96	3.32	7.48	7.81	10.15*	7.21
120	7.48	8.78	11.58	8.54	6.93
144	7.81	8.43	9.49	12.29	4.90

1 Expressed as standard deviations, s.

2 In grams.

* Differs significantly from placebo group.

Table 7

Interindividual Variability[1] of Food Intake[2]: Pentobarbital

Time after administration (Hours)	Dose (mg/kg)				
	0	5	10	20	40
24	8.54	13.19	5.20	11.45	13.82
48	10.58	12.61	13.75	14.07	14.87
72	7.48	16.12	13.08	13.23	15.84*
96	14.04	12.08	9.22	12.57	14.25
120	11.83	18.36	10.86	11.09	10.15
144	16.16	12.61	10.91	11.79	12.45

[1] Expressed as standard deviation, s.

[2] In grams.

* Differs significantly from placebo group.

closer examination of the two sets of data does suggest a possible consistency in the pentobarbital series which does not appear in the Librium data: during the first four days standard deviations for the 40 mg/kg measures are greater than those for measures at any other dose level. As will be shown in the pharmacological data reported later, this dose was well above the threshold for effects on motor coordination and startle and pain response. It may have been that this dose was also very close to the threshold for changes in this consummatory response, changes which may be evidenced first by increased variability and, later, by alterations in level of performance. This kind of possible threshold effect is worthy of specific study.

C. Water intake

Measurement of water intake provided a means of quantifying another appetitive drive: thirst. Detailed records of this consummatory response were kept during the course of several of the present experiments. The results were all similar; those obtained under the identical conditions which held for the food intake measures just described will be reported in detail here.

Research design and procedure. The 5 x 5 Latin square design required five animals in each row, five drug administrations at weekly intervals, and five levels of drug dose. It was identical with that described in detail in the following section. Ten male albino Holtzman rats were used as subjects, being assigned at random to the rows of the design to complete two replications of the same Latin square. All were between 3-4 months of age when the experiment began; none had participated in prior research. All were fed and watered *ad libitum* during the course of the study, the food consisting of standard Wayne Lab Blox pellets for rats and mice.

Water intake was measured daily during the six days between drug administrations as the decrease in weight of the individual water bottle assemblies between the beginning and end of each 24 hour period. The bottles were not allowed to become more than 2/3 empty, and the design of the drinking tip allowed minimal leakage.

Results. Tables 8 and 9 summarize the results of the studies in terms of mean water intake at each of the five dose levels for each drug administered. Gross examination of the tables does not suggest any concommitant variation of a systematic nature between dose levels and water intake. Analyses of variance, summarized in Tables 10 and 11, confirm this impression: it is clear that there is no significant source of variance in dose, in inter-subject variability or in "weeks" or carry-over effects.

To complete the analysis in a way comparable to that used with the food intake data, individual standard deviations, s, were computed for each test session of the two drug experiments; they are reported in Tables 12 and 13. Comparing the s for each drug session with its corresponding s for the placebo condition using the method described in the preceeding section (Senders, 1958), shows significant differences in homogeneity of variance only among those pairs indicated by asterisks in the tables. These appear to be scattered through the tables without any systematic pattern.

Table 8

Mean Water Intake[1]: Librium

Time after administration (Hours)	Dose (mg/kg) 0	4	8	16	32
24	34	37	31	32	35
48	45	35	39	38	34
72	45	37	33	40	30
96	38	42	35	34	29
120	37	41	29	32	36
144	45	45	39	33	38

[1] In grams.

Table 9

Mean Water Intake[1]: Pentobarbital

Time after administration (Hours)	Dose (mg/kg) 0	5	10	20	40
24	29	34	33	38	30
48	31	34	38	34	35
72	31	35	38	34	35
96	37	29	28	36	31
120	32	36	36	34	31
144	37	39	30	37	37

[1] In grams.

Table 10

Water Intake: Librium

Summary of Analysis of Variance

Source of Variance	Sum of Squares	d.f.	Mean Square	F	P
Dose	360.24	4	90.06	---	ns
Subjects	310.64	4	77.66	---	ns
Weeks	792.64	4	198.16	---	ns
Error	4,286.72	12	357.23		
Total	5,750.24	24			

Table 11

Water Intake: Pentobarbital

Summary of Analyses of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
Dose	1,064.56	4	266.14	1.25	ns
Subjects	990.96	4	247.74	1.17	ns
Weeks	368.36	4	92.04	—	ns
Error	2,551.68	12	212.64		
Total	4,975.36	24			

Table 12

Interindividual Variability[1] of Water Intake[2]: Librium

Time after administration (Hours)	Dose (mg/kg)				
	0	4	8	16	32
24	14.97	11.27	7.21*	9.54	9.75
48	15.72	7.55*	10.10	10.00	9.38
72	14.76	9.64	13.27	11.36	11.14
96	15.94	9.80	14.8[illegible]	13.60	16.40
120	12.73	16.76	9.80	7.01	13.60
144	14.49	14.04	5.74	14.32	3.16*

[1] Expressed as standard deviation, s.

[2] In grams.

* Differs significantly from placebo group.

Table 13

Interindividual Variability[1] of Water Intake[2]: Pentobarbital

Time after administration (Hours)	Dose (mg/kg)				
	0	5	10	20	40
24	8.54	8.00	6.86	12.65	10.77
48	8.00	6.71	11.53	10.73	15.68*
72	7.42	10.10	10.15	10.63	11.27
96	14.14	8.25	7.35	14.46	14.83
120	11.83	7.55	14.25	10.68	9.27
144	15.62	12.92	8.72	12.45	13.23

[1] Expressed as standard deviation, s.

[2] In grams.

* Differs significantly from placebo group.

<u>Summary</u>. Before reporting on other experiments in the present series, it may be useful to summarize briefly the main points learned from the studies of consummatory responses just described.

Food and water intake are two measures widely used as indices of appetitive drives. If the drugs affected either or both of these drive processes, one of the effects expected would be modifications in the levels of the respective consummatory response(s). In fact the results of the two experiments failed to provide any evidence for concomitant variation between drug dose levels and the magnitudes of either response within the range of dose levels explored.

However, the drugs had other kinds of significant behavioral effects in which they were distinctly different, i.e., carry-over effects and effects on interindividual or group variability. Librium had significant carry-over effects which were evident in the food intake measure during the period of weekly drug administrations, but pentobarbital did not; no significant carry-over effects appeared in the water intake data. On the other hand, pentobarbital increased interindividual variability in food intake, while Librium did not; neither had any significant effect upon such variability in the water intake measure. These results indicate that, within the range of dose levels studied, various properties of the two behavior patterns were differentially sensitive to the two drugs.

D. Straightaway escape response (SER)

The rate of locomotion in traversing a straightaway to approach food or to escape an aversive stimulus has been used in experimental studies of motivation (e.g., Anderson, 1938; Brown, 1942; Bugelski and Miller, 1938; Buxton, 1941) and of operant conditioning (e.g., Hull, 1934; Graham and Gagné, 1940) where rats have served as subjects. Straightaway operant responses are easily established and they are very stable once they have been acquired. Experience with this type of response during studies of the behavioral effects of an organo-phosphorus anticholinesterase agent (Russell, 1958) suggested that it could be used profitably as a simple prototype of conditioned operant responses and as a means of studying motor output involving locomotor coordination. It has been included in the present series of experiments in two forms: as an approach and as an escape response. Experiments on the latter were more extensive and will be described first.

Research design. A series of three SER experiments were conducted, each involving one of the three prototype drugs. All experiments employed a 5 x 5 Latin square research design with two replications of the same square. The advantage of this design, which makes possible the isolation of a sum of squares corresponding to the particular orders in which the drug under study was administered, was discussed earlier. The Latin square used is shown in Table 14; each replication required five animals, five drug series and five dose levels. Animals were assigned at random to the five rows of the design. Preliminary studies indicated that the measure of performance, rate of traversing the straightaway, returned to an asymptotic level within three to four days, even after administration of the largest drug dose; therefore, one week was set as the standard intertreatment interval. Rats served as subjects in all experiments.

Table 14

Latin Square Design: Straightaway Escape Response

Animal	Week[1]				
	1	2	3	4	5
1,6	0	8	16	32	4
2,7	32	4	0	8	16
3,8	4	16	32	0	8
4,9	8	0	4	16	32
5,10	16	32	8	4	0

[1] Numbers in columns are doses administered, in mg/kg.

<u>Test situation</u>. The conditions required to generate the SER are relatively simple. In general terms, it is necessary to create an environment in which subjects can be exposed to an aversive stimulus and where the only operant escape response possible is to traverse a straightaway path. Previous studies of SER had used electric shock as the aversive stimulus. Since, in the present project, it was intended to use another experimental set up, the classical shuttlebox, to study both escape and avoidance responses to shock, it was considered desirable to add to the number of different forms of motivation included in a final screening battery by using a different aversive stimulus for the SER. Earlier studies (e.g., Braun, Russell, and Patton, 1949) had demonstrated how successfully escape from water could be used to motivate a variety of behavior patterns in the rat, including the SER; therefore, this form of motivation was selected for the present experiments.

Preliminary study of straightaways of different constructions led to a final apparatus consisting of a metal tank 84 inches long, 10 inches wide, and 20 inches deep with a landing platform beginning 12 inches from one end. At the beginning of a test session the tank was filled with water to a depth of 12 inches; the water was maintained between 18° and 20°C. during the session. The apparatus was located in a separate experimental room where such variables as illumination, room temperature and ambient noise were controlled.

A trial consisted of one traversal of the straightaway. The standard operating procedure for this water type of test unit was followed: the animal was placed in the starting end of the straightaway facing the end wall; a Standard Electric timer, calibrated in hundredths of seconds, was started when the experimenter released the animal and was stopped when the animal's forepaws touched the landing platform at the other end. The elapsed time was recorded and later converted to rate, the reciprocal of time, as the basic measure of performance on the trial. Each standard test session consisted of five trials given successively with 30-second intertrial intervals; the median rate for the five trials constituted the measure of performance for a test session.

Subjects. Ten male white rats of the Holtzman albino stock served as subjects for the main experiments. They had not participated in any previous studies, other animals being used for preliminary tests during the development of the apparatus and procedure. They were randomly divided into two groups required for the two replications of the Latin square design and were assigned randomly to the five rows of the design. In order to study the practical problem of repeated use of the same subjects in tests of more than one chemical agent, the same ten animals served as subjects in studying the effects of Librium, pentobarbital and ethanol on the SER. All were housed in separate cages during the experimental period. They were fed and watered ad libitum in their home cages.

Procedure. Since it was desired to measure performance, rather than acquisition, all animals received 175 trials of preliminary training prior to the start of the first drug experiment. Trials 1 to 150 were given 10 per day and the last 25, 5 per day. Throughout this and other studies described in the present report, experimentation went on daily for seven days per week. Information about the stability of the final predrug baseline performance will be presented in the following section describing the experimental results.

The first drug, Librium, was administered orally on the day following completion of preliminary training. Test sessions, consisting of five trials per session, were spaced at 1, 3, 5, 7, 9, 24 hours after administration and then at 24 hour intervals to a total of 144 hours or six days. On the seventh day the animals received their second doses in the Librium series. This weekly regimen continued until each animal had received the five dose levels required by the research design.

Test series for the second drug, pentobarbital, began after an interval of one week during which each animal received five daily "baseline" trials. The same timing and sequence of administrations and test sessions as in the Librium series was used, the five drug levels being: 0, 5, 10, 20 and 40 mg/kg. A third test series followed in turn with ethanol as the chemical agent administered orally at dose levels of 0.00, 0.25, 0.50, 1.00, and 2.00 ml/kg.

Results. Data collected by this procedure provided information about several basic features of the drug-behavior interactions studied. Each of these features will be considered separately in reporting the results of the three experiments.

1. Stability of baseline performance. The research design made it possible to use each animal as its own control. This required that stable predrug baseline performance levels be established. Any changes induced by administration of a drug dose could then be expressed as a per cent of each individual subject's own baseline performance. The first two columns in Table 15 show the means, $\bar{x}$, and standard deviations, s, for each of the 10 subjects, calculated from the performance rates for the 30 trials of the six days immediately preceeding the first drug series. Six, rather than some other number of days during the preliminary training period, were chosen for the calculations because this is the same duration as the 6-day period of each drug series. As the s-column clearly shows, the variability of the distribution of performance rates for each individual subject was very small. For even the most variable animal, No. 4, 95 per cent of the performance rates fell within a range of 0.06; in all cases the mean rates were many times their standard errors.

Estimates were obtained of the internal consistency of the five measures in each of the six test sessions using a form of the conventional split-half method. Because of the number of cases involved, 10, the rank-order correlation, r_o, (Senders, 1958) was used for estimating relationships between measures on odd and even trials. The correlations obtained were 0.88, 0.90, 0.79, 0.88, 0.85, and 0.93, all of which are significant at better than the .01 level of confidence.

Table 15

Straightaway Escape Response: Baseline Performance Levels, Librium

	Rate						
	Predrug[1]		Drug Series[2]				
Animal	$\bar{X}$	s	1	2	3	4	5
1	0.23	0.008	0.19*	0.20*	0.19*	0.19*	0.19*
2	0.22	0.011	0.23	0.24	0.24	0.22	0.23
3	0.21	0.010	0.21	0.19	0.19	0.21	0.19
4	0.21	0.015	0.22	0.20	0.20	0.20	0.19
5	0.18	0.013	0.18	0.16	0.15	0.19	0.17
6	0.20	0.008	0.20	0.19	0.20	0.19	0.17*
7	0.20	0.008	0.20	0.20	0.21	0.21	0.21
8	0.22	0.012	0.22	0.21	0.22	0.19	0.18*
9	0.24	0.014	0.24	0.24	0.23	0.22	0.21
10	0.21	0.009	0.21	0.20	0.21	0.17*	0.17*

[1] Based upon six predrug test sessions.

[2] Based upon last two test sessions of each drug series

* Below two standard deviations from the mean in the distribution of predrug scores.

It would be expected that the effects of drugs at the present relatively low doses would be evidenced as reversible changes in any behavior measures affected. If the preliminary study referred to earlier was valid, reversible changes in behavior should occur within the six days between drug administrations, i.e., performance rates should diminish in magnitude and then return to predrug baseline levels. To check on this recovery, each animal's performance rates were computed for the last two test sessions of each drug period. These data for the Librium series are reported in the last five columns of Table 15; the asterisks indicate those rates which fall beyond ± 2 standard deviations from the means of the distributions of predrug baseline measures, i.e., were significantly different at the .05 level of confidence. The most obvious feature of the data is that the vast majority of performance rates during these last two test sessions had returned to predrug baseline levels, i.e., did not differ significantly from the predrug means. A second feature of the data is that significant differences occurred more frequently at the end of the fifth drug session than at the end of any other session; in all instances the deviations were in the direction of lower performance rates. A third feature worthy of attention from a methodological point of view is the case of animal no. 1, all of whose recovery levels were significantly lower than its predrug level. This suggests that the animal's "baseline" performance rate shifted to a new level following the first drug administration and remained consistently at the new level during the remainder of the experiment. The reason for the shift is unknown, but, the fact that it occurred even when experimental conditions remained constant, means that the possibility of changes of this kind should be anticipated in experiments in which subjects are used as their own controls: shifts in predrug or "control" baselines could lead to misinterpretations of what might appear to be continuing drug effects.

2. Dose-response relations. Relations between dose levels of the drugs and changes in performance of the SER are shown graphically in Figures 1 to 3; the data upon which the curves in these figures are based are presented in Tables 16 to 18. Each point in the figures and each number in the tables is the mean for the performance of 10 animals. Each mean is based upon per cent change in predrug baseline performance computed for each animal individually by dividing its performance rate during a particular test session of a drug series by its baseline rate during the predrug series discussed above. In this way the effect on the SER of each dose level of each drug was evaluated in terms of each animal's own performance under non-drug conditions.

Table 16

Straightaway Escape Response: Effects[1] of Librium on Performance Level

Time after administration (Hours)	Dose (mg/kg) 0	4	8	16	32
1	95	90	84	83	75
3	96	93	90	85	80
5	95	95	87	90	82
7	94	97	91	89	79
9	96	96	92	86	83
24	96	93	92	91	83
48	97	95	96	96	94
72	93	90	90	94	92
96	91	95	94	92	94
120	93	93	96	95	96
144	91	92	96	96	92

1 Expressed as per cent of predrug baseline performance.

Figure 1. Straightaway Escape Response (SER): Librium.

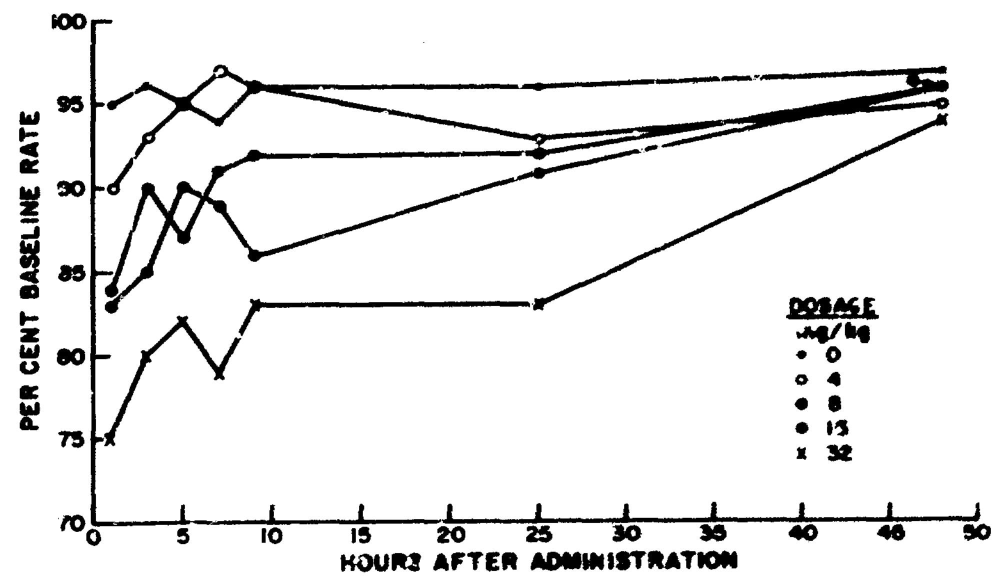


Table 17

Straightaway Escape Response: Effects[1] of Pentobarbital on Performance Level

Time after administration (Hours)	Dose (mg/kg) 0	5	10	20	40
1	93	94	90	82	63
3	94	93	93	93	77
5	98	97	99	97	85
7	97	95	97	99	96
9	95	95	95	94	94
24	93	98	97	95	101
48	95	97	91	94	95
72	92	93	93	92	95
96	95	94	94	92	97
120	89	90	92	96	96
144	89	95	93	98	96

[1] Expressed as per cent of predrug baseline performance.

Figure 2. Straightaway Escape Response (SER): Pentobarbital.

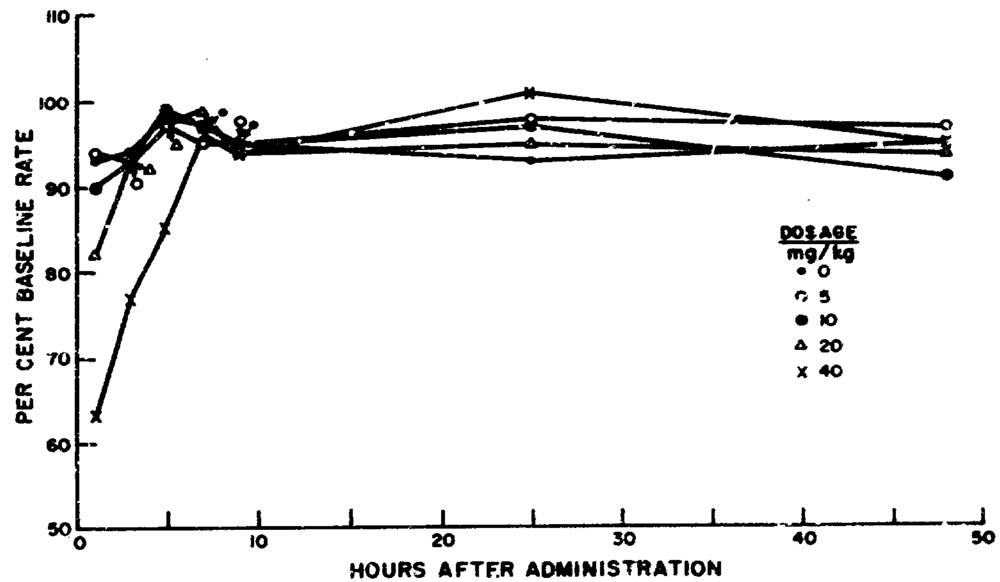


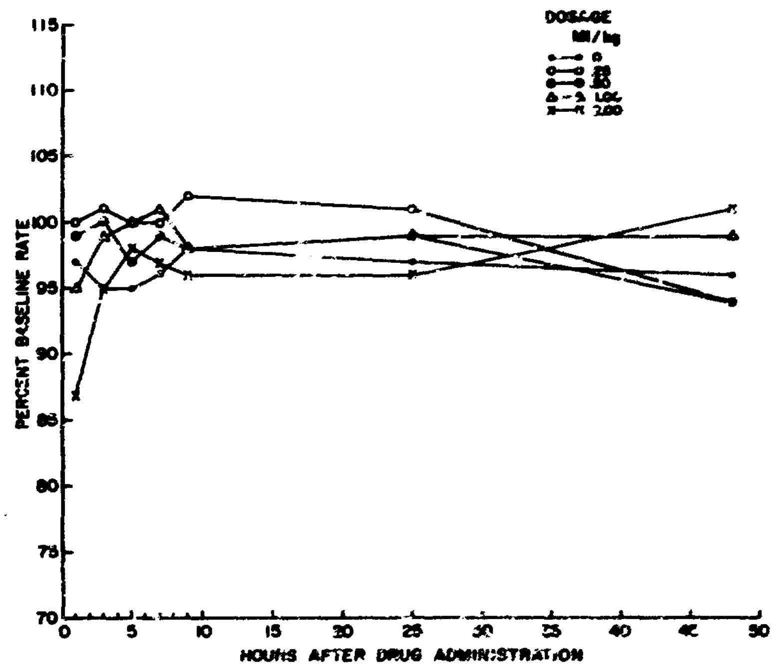
Table 18

Straightaway Escape Response: Effects[1] of Ethanol on Performance Level

Time after administration (Hours)	Dose (ml/kg)				
	0	.25	.50	1.00	2.00
1	97	100	99	95	87
3	95	101	100	99	95
5	95	100	97	100	98
7	96	100	99	101	97
9	98	102	98	98	96
24	97	101	99	99	96
48	96	94	94	99	101
72	95	97	98	101	99
96	101	95	97	100	99
120	99	100	99	95	97
144	100	99	100	101	99

1 Expressed as per cent of predrug baseline performance.

Figure 3. Straightaway Escape Response (SER): Ethanol.



In Figures 1 to 3 SER performance levels at different drug dosages are plotted as a function of time after administration of the drug. Such plots contain information about both dose- and time-response relations. Examination of the curves at any particular point on the abscissa shows the relative effects of different doses at that time. These effects appear most clearly at the test session one hour after drug administration. All three drugs are seen to have produced decrements in the SER at least at some of the dose levels, the greatest effect in absolute terms being for pentobarbital and the least for ethanol. Of greater interest, however, is the direct relation which is shown between the magnitude of decrements when they do occur and the dose levels which produced them: as dose level increased, decrements increased.

The significance of these dose-response effects at the peak effect time were tested by analyses of variance and are given under "doses" in the summary Tables 19 to 21. All effects at this interval were highly significant for the three drugs studied; in each case the results of the analysis make it possible to reject the null hypothesis that the SER measures at the five dose levels are drawn at random from the same population of measures. Therefore, further comparisons among the dose levels were in order. The comparisons of greatest interest are those providing information about which of the dose levels produced significant decrements in SER performance. There are several ways in which such comparisons could be made statistically. Table 22 summarizes the results of analyses using t-tests for correlated observations, since the comparisons involve pairs of measurements on the same subjects. The t-tests are used here to elaborate the results of the analyses of variance and not to contradict them (Senders, 1958). Examination of Table 22 shows that all the Librium doses produced significant decrements in the SER and that the significant effects of pentobarbital and of ethanol can be attributed to dose levels at the high ends of the dose ranges selected for use in the present studies.

Table 19

Straightaway Escape Response: Librium

Summary of Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
Orders	399.88	4	99.97		ns
Error (a)	807.50	5	161.50		
Doses	2,471.28	4	617.82	23.42	<.005
Weeks	343.88	4	85.97	3.26	<.05
Error (b)	844.04	32	26.38		
Total	4,866.58	49			

Table 20

Straightaway Escape Response: Pentobarbital

Summary of Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
Orders	1,955.28	4	488.82	4.42	ns
Error (a)	552.40	5	110.48		
Doses	7,962.08	4	1,990.52	17.80	<.005
Weeks	621.88	4	155.47	1.39	ns
Error (b)	3,577.64	32	111.80		
Total	14,669.28	49			

Table 21

Straightaway Escape Response: Ethanol

Summary of Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
Orders	1,619.30	4	404.83	1.10	ns
Error (a)	1,832.70	5	366.54		
Doses	1,133.90	4	283.48	9.89	<.005
Weeks	366.90	4	91.73	3.20	<.05
Error (b)	918.40	32	28.70		
Total	5,871.20	49			

Table 22

Straightaway Escape Response: Dose-Response Relations[1]

Summary of t-Tests

Dose Levels (mg/kg)	Librium t	Librium P	Dose Levels (mg/kg)	Pentobarbital t	Pentobarbital P	Dose Levels (ml/kg)	Ethanol t	Ethanol P
ovs 4	1.95	<.05	ovs 5	0.17	ns	ovs .25	1.32	ns
ovs 8	3.55	<.005	ovs 10	1.03	ns	ovs .50	1.01	ns
ovs 16	6.29	<.005	ovs 20	5.90	<.005	ovs 1.00	0.86	ns
ovs 32	7.06	<.005	ovs 40	9.84	<.005	ovs 2.00	4.98	<.005

[1] At peak effect time.

One of the reasons for using the present type of replicated Latin square design was to determine whether or not the order in which the various levels of drug dose were administered had an effect upon changes in the SER. Five different orders were used; their possible effects were evaluated by analysis of variance. The results of the evaluation are summarized under "orders" in Tables 19 to 21. It will be seen that no significant F-ratios were found for any of the three drugs, indicating that the order in which the various doses were administered had no systematic effects upon the measures of SER.

3. Time-response relations. Figures 1 to 3 and Tables 16 to 18 show time-response, as well as dose-response, relations for effects of the various drug treatments. The general trends are the same for all drugs studied: peak effects are rapidly attained, i.e., in about one hour, after drug administration and are followed by more gradual recovery. These time characteristics have two features which are helpful in defining them and useful for other purposes to be discussed later: peak effect time and duration of effect. Both measures are given in Table 23 for the results obtained in the present experiments.

Peak effect time is defined as the time after administration of a drug when its maximum effects are achieved. Peak effect times for all three drugs at all dose levels in the present experiment were evidenced at the time of the first test session, one hour after drug administration. Because tests were not made at other times in the interval around one hour, the one-hour figure must be taken as an approximation. In all instances recovery was clearly in process by the three-hour test session.

Duration of effect is defined in terms of hours after administration needed for significant changes in behavior to return to the placebo baseline levels. Table 23 lists these times for those doses which produced statistically significant effects on the SER. Each duration was obtained by

Table 23

Straightaway Escape Response: Peak Effect Times[1] and Durations of Effects[2]

Librium			Pentobarbital			Ethanol		
Dose[3]	Peak	Duration	Dose[3]	Peak	Duration	Dose[4]	Peak	Duration
0	---	---	0	---	---	0	---	---
4	1	3	5	---[5]	---	.25	---[5]	---
8	1	7	10	---[5]	---	.50	---[5]	---
16	1	48	20	1	3	1.00	---[5]	---
32	1	48	40	1	7	2.00	1	3

[1] Hours after administration.

[2] Hours to return to placebo baseline level.

[3] In mg/kg.

[4] In ml/kg.

[5] Not significantly different from placebo group.

determining the test session at which differences between measures under a particular drug condition and measures for its corresponding placebo condition ceased to be significant. t-tests for correlated observations (Edwards, 1958) were used to compute significance. As in the case of statistics for peak effect time, the determination of duration was dependent upon the timing of the test sessions and, therefore, is an approximation; more exact determination would require data obtained from additional test sessions at times immediately preceeding those given in Table 23. The most accurate statement about each statistic on duration presented in the table is that recovery from significant changes in SER occurred within the time period given. It will be seen, as might be expected, that the duration of effects varied from drug to drug and with the dose level administered.

4. Median behavioral dose (BD_{50}). Sciences, e.g., pharmacology and toxicology, concerned with drug effects on biological systems often express dose-response interactions in terms of a relation between dose level and percentage of subjects responding. Such relations have been found to fit two general classes of curves, sigmoid or ogival and hyperbolic (Drill, 1958), although there are relatively few drugs in the latter class. By plotting dosage-mortality curves on log-probability coordinates, a median lethal dose of LD_{50} may be determined (Bliss, 1935 and 1938). When responses other than death are involved, a median effective dose or ED_{50} may be determined in a similar manner. When the response is a behavior pattern, parameters of which can be measured quantitatively, an ED_{50} can be computed. This value represents the dose of a particular drug necessary to produce a significant change of the behavior in 50 per cent of the sample studied. In order to identify such an index as involving behavior, it is useful to refer to it as a median behavioral dose, or BD_{50} (Russell, 1960). BD_{50}'s have been computed from the data provided by the present experiment.

One of the technical questions which arises in determining BD_{50}'s concerns whether or not a particular change in behavior is "significant". The question has been put in another way: is the animal a "positive responder" under the drug conditions existing at the time the behavior is measured? The definition of a significant change or positive response involves a comparison between measures of predrug behavior and behavior under drug conditions. If the predrug baseline is so stable that intraindividual variability is zero, any change under drug conditions is "significant"; such stability may occur, for example, in certain measures of operant conditioning when reinforcement of the response has been manipulated to create it. However, it is much more frequently the case that, although pretraining has led to a high degree of stability, intraindividual variability in predrug performance has not been reduced to zero. Under such circumstances, significance may be defined statistically in terms of the distribution of changes in measures of baseline performance. The procedure followed in the present studies has been to calculate the mean level and the variability of each subject's performance during the six days just preceeding the drug test in question and to establish the magnitudes of the behavior measure which define two standard deviations on either side of the mean; any measure of behavior at the peak effect time of drug action which fell outside these limits was considered as representing a significant change or a positive response. In a normal curve, these limits cut off approximately 95 per cent of the area under the curve; the present definition requires that a change in behavior must deviate beyond this area of normal performance measures in order to be "significant".

Using this statistical definition in analyzing data from an experiment on drug-behavior interactions, relations between the independent and dependent variables, dosage and behavior, may be expressed graphically by plotting percent of subjects showing significant changes in behavior against dose level. The typical plot for dosage-mortality relations is sigmoidal in shape. For convenience of analysis it has become conventional to convert such curves to straight lines by transforming dosages to logarithms and percentages to a probit function of the normal probability curve. With large samples all observations tend to fall on or close to a single straight line, but with small samples the variation is greater and values for individual observations should not be weighted equally in computing the best-fitting curve. Tables for corrected probits have been provided (Bliss, 1938).

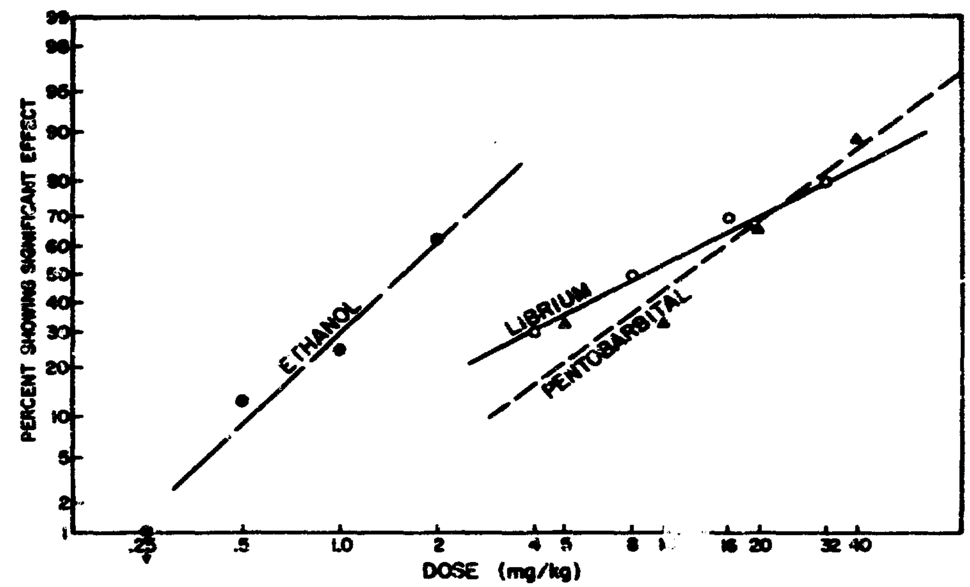
This method of probit analysis was applied to data from the three SER experiments, the straight lines being drawn by best visual fit. The resulting BD_{50}'s were 9.0 mg/kg for Librium, 11.5 mg/kg for pentobarbital, and 1.5 ml/kg for ethanol, as shown in Figure 14.

Data such as these may be fitted more precisely. Karber's Method for calculating the dose-response curve is a special case of the maximum likelihood method of Bliss and assumes that drug effects are distributed according to log dose, rather than dose, of drug. As an example of this second approach Cornfield and Mantel's (1950) modification of the Karber Method was applied to the non-graphical calculation of the BD_{50} for the Librium data. The calculated value of 9.2 mg/kg compares very closely with the graphic log-probit plot value of 9.0 mg/kg reported above.

Both of these methods also allow for the determination of the variance or standard error of the BD_{50}, but are relatively time-consuming to apply. It is anticipated that a procedure which allows calculation of the standard error of the BD_{50} directly from the log-probit plot will be more feasible for routine screening applications.

5. Variability of performance. Chemical agents may have their main effects by altering the variability of behavior. It is conceivable that the mean level of a particular performance might remain essentially unchanged after administration of an agent, yet the variability in performance of subjects affected might increase to an extent which seriously influenced the predictability of their behavior at any given time and thus disorganized their normal operations as members of a group. The present experiments provided information about three different kinds of behavioral variability.

Figure 4. Dose-Response Curves for the SER Behavioral Test: Librium, Pentobarbital, and Ethanol.



a. Intraindividual variability during a single test session. Individuals characteristically vary in their performance from trial to trial, even when the conditions generating the behavior are held as objectively constant as possible. Such intraindividual variability tends to decrease as the behavior pattern is learned and overlearned. In the present experiments it is to be expected that this kind of variability would attain its minimum values when a stable baseline of performance had been reached during predrug test sessions. Following administration of a chemical agent variability might be altered, returning to baseline levels during the subsequent recovery period.

In order to study this kind of intraindividual variability it is necessary to have more than one measure of performance during each test session. Whether this is possible depends upon the nature of the behavior measured and, therefore, upon the conditions under which it is generated. Some but not all of the responses studied in the present project met this criterion; SER was one and can be used to illustrate the method of analyzing data for evidence of drug effects on this kind of variability.

The five trials given during each test session constitute a distribution of measures from which standard statistics on central tendency and variability may be calculated. For present purposes a standard deviation, s, was computed for each subject at the peak effect time following each drug administration, using the original time measures taken during each trial. The standard deviation, then, constituted a "score" for the subject's variability of performance and could be compared with its analogous scores under other treatment conditions or with scores obtained by other subjects. Table 24 gives the means of the individual standard deviations for the group of subjects as a whole. There appears to be no systematic relations between variability and dose level, with the possible exception of the pentobarbital data where variability increased appreciably at the two high dose levels. Analyses of variance, summarized in Table 25, support this general impression: no significant F-ratios were obtained except in the case of pentobarbital dose effects.

Table 24

Straightaway Escape Response: Intraindividual Variability at Peak Effect Time

Librium		Pentobarbital		Ethanol	
Dose	Mean s	Dose	Mean s	Dose	Mean s
0	.22	0	.50	0	.38
4	.43	5	.44	.25	.28
8	.46	10	.48	.50	.30
16	.39	20	.81	1.00	.49
32	.53	40	1.81	2.00	.44

Table 25

Straightaway Escape Response: Intraindividual Variability at Peak Effect Time

Summary of Analyses of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
		A. Librium			
Dose	0.43	4	0.11	---	ns
Subjects	1.43	9	0.16	1.27	ns
Error	4.53	36	0.13		
Total	6.39	49			
		B. Pentobarbital			
Dose	13.39	4	3.35	7.66	<.01
Subjects	5.44	9	0.60	1.38	ns
Error	15.73	36	0.44		
Total	34.56	49			
		C. Ethanol			
Dose	0.34	4	0.08	2.15	ns
Subjects	0.63	9	0.07	1.81	ns
Error	1.40	36	0.04		
Total	2.37	49			

The results with Librium and ethanol illustrate the general case in which level of performance is more sensitive to drug effects than is intraindividual variability of the performance. The fact that the mean standard deviations for the lower doses of pentobarbital remained at very nearly the same level or plateau and that a significant increase in variability occurred between 20 mg/kg and 40 mg/kg suggests the existence of a "threshold" effect within the latter range. As will be pointed out in section V below, 40 mg/kg of pentobarbital is a relatively high dose for studies of behavior since this is at a level where hypnotic effects begin to appear and thus confound other effects the drug may be having upon behavior. If all chemical agents had characteristics similar to the three drugs studied in the present project, changes in level of performance are, for practical screening purposes, likely to be more sensitive measures of chemical effects than are measures of intraindividual variability.

b. Intraindividual variability during a test series. Another kind of intraindividual variability is evidenced in comparisons of each subject's performance over two or more test sessions. The present experiments were designed to minimize this variability during the predrug phases; this is what is meant by the "stability" of the behavior patterns studied. Intraindividual variability during a drug test series would be affected significantly if the drug produced changes from the level of predrug baseline performance. Clearly this is a description of the same phenomenon as that considered in the earlier discussion of effects of drugs on level of performance. This being the case, no useful purpose seems to be served by using both types of analysis.

c. Interindividual variability during test sessions. Characteristically individuals differ from each other in the levels at which they perform the same task, even when intraindividual differences are minimized. It is possible that an effect of the administration of chemical agents is an alteration in such interindividual differences. The effect would be evidenced by changes in the spread of mean SERs for individual subjects during the drug period as measured, for example, by the magnitude of the standard deviations at each dose level. To test the possibility that this effect operated under the present experimental conditions, standard deviations were computed for performance at all dose levels, using the standard per cent change from predrug baseline as the performance measure. The results are summarized in Tables 26 to 28. The significance of the difference between variance at any of the drug levels and variance at the corresponding placebo levels may be tested by comparing the two unbiased estimates of the population variances as an F-ratio (Senders, 1958). When such ratios were computed for the data in Tables 26 to 28, none was found to approach significance in the Librium series. Some significant changes were found in the pentobarbital and ethanol data, being concentrated in the period immediately following drug administration and then at the high dose levels. In these particular instances the drug is shown to have increased the spread of differences between individual subjects. Such an effect may be important in the sense that it may interfere with, or "incapacitate", the normal functional relations among individuals in a working group, thus decreasing both the quality and quantity of the group's productivity.

Table 26

Straightaway Escape Response: Effects of Librium on Interindividual Variability of Performance[1]

Time after administration (Hours)	Dose (mg/kg)				
	0	4	8	16	32
1	6.32	6.56	8.37	8.37	9.11
3	9.06	8.72	7.75	7.48	12.25
5	7.48	9.95	9.85	8.19	8.12
7	7.00	10.92	11.66	5.74	9.22
9	6.00	8.66	7.14	6.48	10.49
24	7.62	8.54	6.32	5.83	8.19
48	6.86	8.43	7.28	9.17	11.31

[1] Expressed as the standard deviation, s.

Table 27

Straightaway Escape Response: Effects of Pentobarbital on Interindividual Variability of Performance[1]

Time after administration (Hours)	Dose (mg/kg) 0	5	10	20	40
1	5.92	10.63	12.96[2]	8.43	26.14[2]
3	7.68	9.54	11.66	9.38	20.25[2]
5	7.62	12.96	10.86	9.43	10.54
7	10.82	10.68	12.25	6.86	13.27
9	8.[illegible]4	12.92	10.63	10.15	10.39
24	10.10	9.95	10.68	13.93	10.00
48	8.[illegible]3	10.58	10.72	11.31	14.07

[1] Expressed as the standard deviation, s.

[2] Significant at P = .05 or better when compared with the variance of the corresponding placebo group.

Table 28

Straightaway Escape Response: Effects of Ethanol on Interindividual Variability of Performance[1]

Time after administration (Hours)	Dose (ml/kg)				
	0	.25	.50	1.00	2.00
1	9.49	7.35	8.37	15.26	14.35
3	6.32	6.48	12.00	10.44	16.58[2]
5	7.68	6.78	9.90	7.48	14.11[2]
7	6.32	10.63	7.75	8.83	16.28[2]
9	6.63	9.27	9.17	9.43	14.76[2]
24	9.27	5.00	8.37	11.36	15.62
48	7.21	15.81	8.12	8.00	11.40

[1] Expressed as the standard deviation, s.

[2] Significant at P = .05 or better when compared with the variance of the corresponding placebo group.

6. Carry-over effects. The case was stated earlier for the practical importance to a program for the screening of chemical agents of the repeated use of the same subjects whenever possible; for all but the very simplest measures of behavior the time and effort required to establish stable baselines of performance are considerable. Repeated uses of the same subjects does, however, introduce complications in the interpretation of results, complications arising from carry-over effects which may be based upon pharmacological, biochemical, or behavioral factors.

Pharmacologically a chemical agent is considered to have a "...cumulative action when its elimination and/or catabolism are relatively slow and the full response is the result of the summation of 2 or more doses (Krantz and Carr, 1961)". This definition emphasizes the continuing presence in the body of dose 1 when dose 2 is administered; carry-over effects are due to interaction between the two doses.

Cumulative effects may also occur when the after-effects of dose 1 on some biochemical system in the body persists at the time dose 2 is administered, even though the drug in dose 1 is no longer present. The effects of reserpine on serotonin and norepinephrine, persisting over days when the drug itself is eliminated or catabolized in hours, is an example (Russell et al, 1962).

In a third instance, carry-over effects may result from the mere procedure of repeated measurements of the same behavior pattern. The process of learning obviously involves such effects. It is possible that performance after administration of a chemical agent may produce behavioral effects which persist after the agent has been completely eliminated or metabolized. An experiment by Behar and Riopelle (1957) illustrates this possibility by demonstrating a persistent retardation in the acquisition of a conditioned avoidance response following a period of trials under the drug reserpine; the authors suggest that "...some new adaptive response seems to be acquired during the drug phase which persists into Phase II".

The Latin square designs used in the present experiments made it possible to calculate sums of squares for successive test series and from this to determine significances of carry-over effects. Preliminary results using the return of behavior measures to predrug baseline levels suggested that seven days constituted an adequate interdose interval to protect against carry-over effects. That return to baseline performance level was in fact an inadequate criterion upon which to base the decision is shown in Table 29, which summarizes the pertinent parts of analyses of variance for carry-over effects. Comparison of sums of squares for successive administrations of the drugs, "weeks", with the proper error term, "error (b)", (Edwards, 1960) resulted in significant F-ratios for two of the three experiments, indicating that carry-over effects did occur during the Librium and ethanol drug series but not during the pentobarbital series.

What can be said about the probable bases for the carry-over effects? The possibility that unusual behavioral effects produced during the drug test sessions persisted after the agents were no longer present in the body seems an unlikely candidate. The standard procedure for the present experiments required sufficient predrug training to establish stable baselines of performance. With these baselines as bench marks it was possible to determine whether particular behavior patterns did in fact return to baseline by the end of the interdose intervals. Such return would be a contraindication to the possibility of persisting new responses of the Behar-Riopelle variety. Evidence was presented earlier that, following even the highest drug doses, the SER recovered to placebo-condition levels by 48 hours.

In order to test the possibilities that the carry-over effects may be attributed to the persisting presence of the drugs within the body or to effects of the drugs on biochemical systems which take time to recover, information is needed on the time characteristics of drug absorption-metabolism and of changes in biochemical systems affected. This is one of the reasons for including pharmacological studies in the present project. The results of the studies are reported in section V below, where the question of the bases for the carry-over effects will be discussed further.

Table 29

Carry-Over Effects: Summary of Analyses of Variance for SER Experiments

Drug Series	Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
Librium	Test Series (Weeks)	343.88	4	85.97	3.26	<.05
	Error (b)	844.04	32	26.38		
Pentobarbital	Test Series (Weeks)	621.80	4	155.47	1.39	ns
	Error (b)	3,577.64	32	111.80		
Ethanol	Test Series (Weeks)	366.90	4	91.73	3.20	<.05
	Error (b)	918.40	32	28.70		

7. Dose-order effects. It might be expected that any carry-over effects arising from the presence of a chemical agent or from its after-effects on a biochemical system(s) would be some function of the dose levels involved. For example, a high dose might be associated with greater cumulative effects than a low dose and/or with a longer duration of cumulative effects. If this were the case, it would be a reasonable hypothesis that carry-over effects, when present during repeated drug administrations to the same subjects, might be some function of the sequence or order in which the various doses were administered. For example, it might be predicted that higher doses given early in a sequence would show greater carry-over effects than lower doses given early.

By using a Latin square research design involving replication of the same square it was possible to isolate a sum of squares corresponding to the particular sequences or orders of administration of the various doses employed. This "orders" sum of squares could then be used to test whether the sequence of administration did in fact produce significant effects upon the behavior patterns measured. Table 30 summarizes the analyses of variance for the SER measures at their peak effect times. In none of the three experiments were the dose-order effects significant.

Summary. The experiments on the straightaway escape response were planned to examine some of the basic issues which affect behavioral screening programs involving repeated use of the same subjects. The results of the studies are summarized below. Three experiments were conducted; all used rats as subjects. The chemical agents used were Librium, pentobarbital and ethanol; all were administered orally.

1. The SER was shown to be an easily-established behavior pattern, which could be maintained at a highly stable baseline level following its acquisition. When affected by one of the chemical agents used in the present project, the SER always underwent the same pattern of change, consisting of a rapid decrement in the efficiency of the response, i.e., slower responding, followed by a more gradual recovery to predrug baseline levels.

Table 30

Dose-Order Effects: Summary of Analyses of Variance for SER Experiments

Drug Series	Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
Librium	Orders	399.88	4	99.97	0.62	ns
	Error (a)	807.50	5	161.50		
Pentobarbital	Orders	1,955.28	4	488.82	4.42	ns
	Error (a)	552.40	5	110.48		
Ethanol	Orders	1,619.30	4	404.83	1.10	ns
	Error (a)	1,832.70	5	366.54		

2. The magnitude of the change at peak effect time was found to be very significantly related to the dose of drug administered: as dose level increased, decrements increased. This relation operated after a threshold for change in the SER had been reached; below that threshold, changes in drug dose level produced no effects on the response.

3. For all three drugs studied, peak effects were rapidly attained, i.e., in about one hour, after drug administration. Above the threshold dose required to produce a change in the SER, the duration of the effects varied systematically with the drug dose administered: duration increased as dose level increased.

4. A median behavioral dose, BD_{50}, was determined for each of the three drugs studied. A statistical technique is described for deciding whether or not a change in an individual subject's SER was "significant". Using this definition, dosage-response curves were plotted on log-probability coordinates to determine the BD_{50}'s, which were 9.0 mg/kg for Librium, 11.5 mg/kg for pentobarbital and 1.5 ml/kg for ethanol.

5. Chemical agents may produce significant changes in the variability of performance as well as in the level of performance. Two kinds of variability were studied:

a. Intraindividual variability within single test sessions showed significant drug effects only in the pentobarbital experiment and then only at high dose levels where hypnotic effects of the drug may have been an important influence.

b. Interindividual variability within single test sessions is evidenced by changes in the standard deviation of the distribution of mean SER's for individual subjects. In the present experiments such changes appeared as increases in the spread of differences among individual subjects. Librium produced no such effects; some significant changes were found in the pentobarbital and ethanol series, being concentrated in the period immediately following drug administration and then at the high dose level.

6. The research design used in the three experiments was a 5 x 5 Latin square with two replications of the same square. This design was selected in order to test its usefulness in identifying the occurrence of carry-over and sequential effects in research involving repeated administration of chemical agents to the same subjects.

The replicated Latin square design made it possible to determine whether or not the order or sequence in which the various levels of drug dose were administered had an effect upon changes in the SER. No such effects were found in any of the three experiments.

Carry-over effects from one drug administration to another may introduce complications in the interpretation of results. This may be a chronic problem of considerable practical importance in behavioral screening programs where the same animals are used for extended periods of time during which drug administrations include not only different doses of the same agent, but also agents with different chemomorphologies. The present experiments demonstrated that significant carry-over effects may or may not occur. They were found during the Librium and ethanol series, but not during the pentobarbital series. With adequate research designs it is at least possible to determine whether such effects have occurred in order that they may be considered in interpreting results obtained, even though they have not been eliminated.

E. Straightaway approach response (SAR)

The straightaway approach response, like the SER, is a simple conditioned operant response, which is easily established and is relatively stable once it has been acquired. In contrast with the SER, it is an operant response motivated by an appetitive drive, rather than a response to escape from an aversive stimulus. Since behavior under these two general types of motivation may be affected differentially by drug action, the SAR was included as another measure in the present battery of behavior tests.

Research design. Two SAR experiments were conducted, each with a different research design and each involving only one of the three prototype drugs.

The first experiment employed a 4 x 4 Latin square design with five replications of the same square. Each replication required four animals, four drug series and four dose levels. Animals were assigned at random to the four rows of the design. As in the SER experiments, the standard interval between drug administrations was one week. Rats were used as subjects and the effects of Librium at doses of 0, 4, 8, 16 mg/kg of body weight were studied.

The second experiment used a randomized groups design (Edwards, 1960) in which $n = 25$ subjects were assigned at random to $k = 5$ dose levels with five subjects for each dose level. Subjects within each dose group were administered their appropriate drug dose only once. The drug studied was pentobarbital sodium at dose levels of 0, 5, 10, 20, and 40 mg/kg. Again, rats served as the subjects.

Subjects. All subjects were male white rats of the Holtzman Albino stock. None had participated in previous experiments. All weighed between 330 and 430 grams. In both studies they were randomly assigned to their respective treatment groups in accordance with the requirements of the research design. All were housed in separate cages during the experimental period and had water available *ad libitum*.

Test situation. In general terms, the test situation consisted of a simple straightaway connecting a starting box and a food box, which the animal had to traverse in order to reach the food incentive. The straightaway was 5 ft. 10½ inches long, 3 3/4 inches wide and 4 inches high. Running times were recorded automatically on a Standard Electric timer calibrated in hundredths of seconds; the timer was activated at the start of each run when the animal interrupted a photobeam at the exit of the starting box and was stopped by interruption of a photobeam at the entrance to the food box. At the beginning of each trial the experimenter placed the animal in the starting box and released it for the run by raising a door which separated the box from the straightaway. The apparatus was located in its own experimental room where illumination, ambient noise and other distracting stimuli could be controlled.

Procedure. The procedure consisted of four major phases.

Since the SAR involved approach to food, a standard food deprivation schedule had to be imposed before the experimental trials began. This schedule is established in Phase 1. After their arrival in the laboratory all animals were kept on *ad libitum* feeding for 16 days. On the 17th. day adaptation to the food deprivation schedule began with a 24 hour period without food. The following day all animals were fed twelve grams of food in the form of standard Wayne Blox for rats. This controlled feeding continued at 24 hour intervals for one week. Daily records were kept of each subject's weight and of its food intake.

Phase 2 consisted of preliminary training in the straightaway, which began on the day immediately following the end of Phase 1. For the first two days each animal was taken from its home cage and placed in the apparatus, being given two minutes of orientation each day in each third of the straightaway. Training on the third day began by putting each animal into the food box for 90 seconds, where it found a supply of sugar pellets of the kind to be used as reinforcement during the later experimental trials. Sugar pellets were used instead of the standard Wayne Lab Blox because rats do not satiate as rapidly with the former as with the latter and because the former do not provide as strong olfactory cues. After 90 seconds in the food box, each animal was given five trials which began in the starting box and ended with entry into the food box. A subject was allowed to remain up to five minutes in any one third of the straightaway, but was forced to move on after this maximum time. If after 15 minutes entry into the food box had not occurred, the animal was removed from the straightaway and the next trial begun. When an animal completed a trial within this time limit, it was allowed 10 seconds in the food box. After the five trials, which constituted a test session, the pellets remaining in the food box were weighed and the animal returned to its home cage, where it received regular diet in the amount of 12 grams minus the amount eaten in the food box. This procedure was followed for 14 days.

Having shaped the SAR by this preliminary training procedure, the task for Phase 3 was to establish stable predrug baselines for the performance of each animal. The standard procedure introduced in Phase 2 was followed: five daily trials constituted a test session; these were run in succession with a 10 second intertrial interval; food intake was controlled as described in the preceeding paragraph. A criterion for establishment of a stable baseline was arbitrarily set: each animal was required to maintain a mean time for the five trials of a test session which did not vary more than 10 per cent during a period of six successive days. There were individual differences in the amount of training necessary to achieve this criterion. Therefore, each individual animal was moved to the next phase of the procedure on the day following that on which it reached the criterion.

Phase 4 was the drug phase. Drugs were administered by intubation in accordance with the research designs described earlier. The administration was so planned in each case that the first behavioral test session could begin 20 minutes later; standard test sessions were then run at 24 hour intervals to a total of 144 hours, i.e., six days, thus providing data for the study of time-response relations. In the experiment involving the Latin square design, animals received their second doses of Librium on the seventh day and this weekly regimen continued until each animal had received the four dose levels required. In the second experiment, all data for the randomized groups design were collected by the completion of the sixth day test session.

Results. The results of applying these procedures were analyzed to provide information about several features of drug-behavior interactions. For the analysis the conventional procedure was followed of converting the raw time measures to rate scores, the reciprocal of time.

1. Stability of baseline performance. In order to use each animal as its own control, it was necessary to establish stable levels of predrug baseline performance. Any changes induced by administration of a drug could then be expressed as a per cent of each subject's own baseline performance. The first two columns in Table 31 present the means, $\bar{X}$, and standard deviations, s, for each of the 20 subjects in the first-Librium-experiment, calculated from the performance rates for the 30 trials of the six days immediately preceeding the first drug series. Table 32 gives comperable data for the 25 animals participating in the second-pentobarbital-experiment.

As in the case of the SER, it would be expected that the effects of the drugs at the present relatively low dose levels would be evidenced as reversible changes in any behavior measure affected. To check on recovery, each animal's performance rates were computed for the last two test sessions of each of the Librium series. These data

Table 31

Straightaway Approach Response: Baseline Performance Levels for Experiment 1 (Librium)

	Rate					
	Predrug[1]		Drug Series[2]			
Animal	$\bar{X}$	s	1	2	3	4
1	0.90	0.03	0.71*	0.82*	0.69*	0.87
2	0.64	0.07	0.68	0.64	0.45*	0.67
3	0.65	0.04	0.64	0.59	0.71	0.66
4	0.62	0.04	0.62	0.46*	0.48*	0.58
5	0.67	0.14	0.79	0.75	0.85	0.70
6	0.72	0.10	0.68	0.65	0.71	0.69
7	0.83	0.04	0.79	0.71*	0.77	0.71*
8	0.96	0.03	1.03*	0.76*	0.88*	0.78*
9	0.83	0.05	0.77	0.69*	0.64*	0.70*
10	0.76	0.08	0.65	0.66	0.67	0.65
11	0.88	0.06	0.78	0.56*	0.68*	0.63*
12	0.88	0.07	0.78	0.90	0.67*	0.67*
13	0.83	0.06	0.82	0.83	0.66*	0.88
14	0.90	0.09	0.83	0.83	0.78	0.94
15	0.92	0.06	0.89	0.81	0.71*	0.88
16	0.98	0.06	1.00	0.95	0.99	0.96
17	0.90	0.11	1.05	0.87	0.94	1.02
18	0.91	0.11	0.98	0.75	0.86	0.98
19	1.01	0.08	1.10	0.94	0.93	0.90
20	0.80	0.23	1.04	0.97	0.94	0.90

[1] Based upon the last six predrug test sessions.

[2] Based upon the last two test sessions of each drug series.

* Beyond ± 2 standard deviations of predrug mean.

Table 32

Straightaway Approach Response: Predrug Baseline Performance Levels for Experiment 2 (Pentobarbital)

Animal	$\bar{x}$	s
1	0.57	0.09
2	0.67	0.16
3	0.61	0.03
4	0.60	0.05
5	0.71	0.02
6	0.80	0.05
7	0.77	0.01
8	0.65	0.04
9	0.61	0.03
10	0.56	0.05
11	0.77	0.04
12	0.84	0.05
13	0.69	0.04
14	0.57	0.02
15	0.64	0.03
16	0.77	0.05
17	0.78	0.09
18	0.75	0.03
19	0.67	0.01
20	0.78	0.07

are reported in the last four columns of Table 31; the asterisks indicate those rates which fall beyond ± 2 standard deviations from the means of the distributions of predrug baseline measures. It will be seen that the majority of these performance rates did in fact return to predrug levels within the six-day intertreatment periods, significant differences occurring more frequently as the series progressed. No comparable data are available for the pentobarbital experiment since the randomized groups design called for only one drug administration to each subject.

2. Dose-response relations. Table 33 presents the basic data relating to dose-response relations for the Librium experiment. Each number in the table is the mean for the performance of 20 subjects calculated from the per cent change from predrug baseline performance for each individual animal. In this way the effect on the SAR of each dose level of the drug was evaluated in terms of each animal's own performance under non-drug conditions. The data are plotted graphically in Figure 5.

Inspection of the table and figure suggest that administration of Librium resulted in decrements of performance which were related to the dose levels which produced them: in general, as dose level increased, decrements increased, although no clearly defined differences appeared at the 4 mg/kg and 8 mg/kg levels. The significance of this dose-response relation at the peak effect time was tested by analysis of variance and is given under "doses" in the summary Table 34. Drug effects at this interval after administration were highly significant; therefore, further comparisons among the dose levels were made using t-tests for correlated observations, since the comparisons involved pairs of measurements on the same subjects. Performances at all three dose levels were found to be significantly different ($P < .01$) from the performance of the placebo group.

Table 33

Straightaway Approach Response: Effects[1] of Librium on Performance Level

Time after administration (Hours)	Dose (mg/kg) 0	4	8	16
20 minutes	95	83	85	55
24	98	92	96	95
48	96	94	91	88
72	95	95	95	92
96	96	93	98	92
120	93	94	101	93
144	93	96	94	94

[1] Expressed as per cent of predrug baseline performance.

Figure 5. Straightaway Approach Response (SAR): Librium.

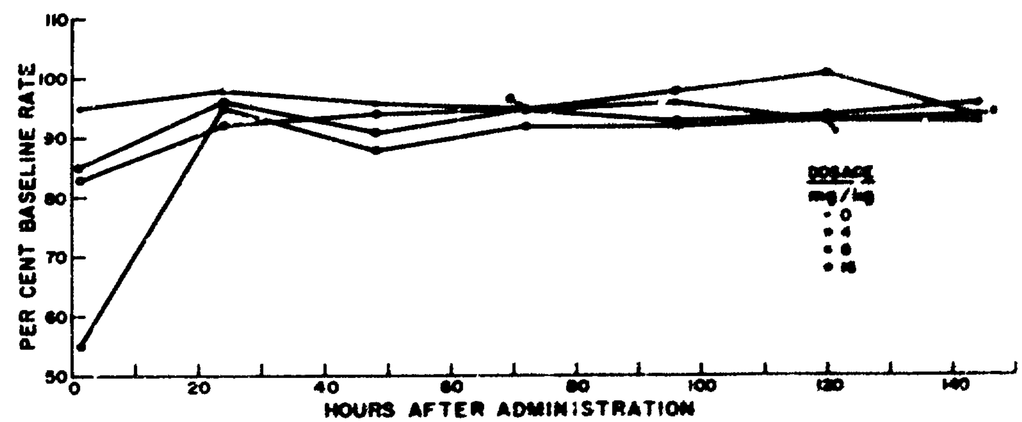


Table 34

Straightaway Approach Response: Librium

Summary of Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
Orders	4,844.94	3	1,614.98	1.3007	ns
Error (a)	19,865.80	16	1,241.61		
Doses	17,743.34	3	5,914.45	15.0365	<.01
Weeks	3,878.84	3	12,929.47	3.2871	<.05
Error (b)	21,240.57	54	393.34		
Total	67,573.49	79			

A different method of analysis was appropriate for treating results of the second SAR experiment, which employed a randomized groups design in which the subjects received only one drug administration each. The mean per cent baseline performance levels for the 0, 5, 10, 20 and 40 mg/kg groups were 119, 102, 79, 47 and 17, respectively. There are obvious decrements in performance which appear to increase systematically as dose level increases. The significance of the decrements was calculated using analysis of variance for the randomized groups design; the result was the highly significant F-ratio shown under "B. Pentobarbital" in Table 35. The table also summarizes the results of treating the data from the first test series of the Librium experiment as if it were an independent randomized groups design. Again the dose effects were found to be significant, a finding confirming the results of the more elaborate Latin square analysis reported in the preceeding paragraph.

3. Time-response relations. To study the time course of a particular drug-behavior interaction requires repeated measurements of the behavior at a number of intervals following administration of the drug. It is important that several of the measurements be made at times when the process of absorption and metabolism of the drug is undergoing its major changes. When the process is a rapid one, it is necessary to schedule several test sessions within a period of less than 24 hours, as, for example, in the SER experiments described earlier. Under these circumstances a technical problem arises when the motivation used in generating the behavior pattern measured is a drive which is satiated by reinforcement of the behavior, e.g., hunger or thirst; the behavior is altered with repeated reinforcements and these motivational changes confound effects which may be produced by the drug. This was a serious problem in the present experiments, where several of the behavior patterns considered important to study were dependent upon satiable appetitive drives.

One solution to the problem requires the introduction into the research design of an additional independent variable:

Table 35

Streightaway Approach Response: Randomized Groups Design

Summary of Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
		A. Librium			
Dose	12,253.75	3	4,084.58	4.10	$<.025$
Error	15,953.20	16	997.08		
Total	28,206.95	19			
		B. Pentobarbital			
Dose	34,074.64	4	8,518.66	27.01	$<.005$
Error	6,308.20	20	315.41		
Total	40,382.84	24			

time after drug administration when the first measurements of behavior are made. By using several times, relations between pharmacological changes within the body and changes in behavior may be mapped. But to carry out such a design either requires additional groups of subjects or additional test sessions for the same subjects, in both instances increasing considerably the time and level of effort involved in completing each experiment.

This preamble explains why, in the present SAR experiments, time-response relations can be described only in approximate terms. Table 36 summarizes the available information. Each duration in the table was obtained by determining the test session at which differences between measures under a particular drug condition and measures for its corresponding placebo condition ceased to be significant; significances were computed using t-tests for correlated observations (Edwards, 1958). As in the case of the SER, the peak effect time was reached very rapidly after administration of Librium at all dose levels. Differences between the drug and placebo groups disappeared within 24 hours. One discrepancy between the SER and SAR appears at the 16 mg/kg dose where the SAR recovered more rapidly than the SER, which did not return to the placebo group's level of performance until after 24 hours.

4. Median behavioral dose. Data from which to determine BD_{50}'s for the SAR experiment are not as complete as is necessary for reliable calculations. More points are needed between the 0 and 100 per cent levels on the response dimension. To obtain rough and very tentative approximations, the log-probit method used in treating the SER data was applied to the results of the Librium experiment, giving a BD_{50} of 7.7 mg/kg. No approximation can be made for the pentobarbital results.

Table 36

Straightaway Approach Response: Peak Effect Times and Durations of Effects, Librium

Dose (mg/kg)	Peak Effect Time[1]	Duration of Effects[2]
0	---	---
4	20	< 24
8	20	< 24
16	20	< 24

[1] Minutes after drug administration.

[2] Hours until return to baseline performance levels.

5. Interindividual variability during test sessions. Individuals differ from each other in the levels at which they perform the same tasks. This interindividual variability may be affected by chemical agents, effects being evidenced as changes in the spread of performance measures during the drug period. To test the possibility that such an effect might have operated under the present experimental conditions, standard deviations were computed for performance at all dose levels, using the standard per cent change from predrug baseline as the performance measure. The results are summarised in Table 37. The table presents information for the Librium experiment only, since the randomised groups design of the pentobarbital experiment did not provide measures of behavior from the same animals at all dose levels.

The significance of the difference between variance at any of the drug levels and variance at the corresponding placebo level may be tested by comparing the unbiased estimates of the population variances as an F-ratio (Senders, 1958). When such ratios were computed for the data in Table 37, it was clear that the increases in interindividual variability during the first two test sessions, 20 minutes and 24 hours, after administration of the drug were highly significant, all F-ratios being well beyond the .05 level of confidence.

6. Carry-over effects. The Latin square design used in the Librium experiment made it possible to test for the significance of any carry-over effects. Comparison of sums of squares for successive administrations of the drug with the proper error term resulted in an F-ratio significant at better than the .05 level of confidence, indicating that carry-over effects did occur. The data from this analysis of variance are summarised under "weeks" in Table 34. The presence of carry-over effects in this Librium series involving the SAR corresponds to similar effects for the action of Librium on the SER.

Table 37

Straightaway Approach Response: Effects of Librium on Interindividual Variability of Performance[1]

Time after administration (Hours)	Dose (mg/kg) 0	4	8	16
20 minutes	9.70	30.94[2]	30.41[2]	25.40[2]
24	11.53	20.20[2]	24.00[2]	23.28[2]
48	12.41	17.52	25.90[2]	17.61
72	24.96	15.81	19.92	12.12
96	18.92	16.52	22.32	13.49
120	18.60	16.22	19.49	17.97
144	16.19	14.53	18.03	18.76

[1] Expressed as standard deviations, s.

[2] Significant at P = .05 or better when compared with the variance of the corresponding placebo group.

7. Dose-order effects. The research design for the present Librium experiment also provided data from which to determine effects, if any, on the level of SAR performance of the particular sequences or orders in which the drug was administered. Analysis of variance was used to test this possibility, the results of the test being summarized under "orders" in Table 34. It will be seen that the F-ratio obtained was not significant.

Summary. The results of the two SAR experiments supplement those of the SER studies. The main issues on which they contribute information are summarized briefly in the following paragraphs. Rats were used as subjects in both SAR experiments. One experiment employed a 4 x 4 Latin square design with five replications of the same square; the other was a simple randomized groups design involving five dose groups with five subjects per group. The former studied effects of Librium on the SAR; the latter, the effects of pentobarbital. The drugs were administered orally.

1. Although it involved a different form of motivation, the SAR had basic characteristics in common with the SER. It was easily established and remained at a very stable level of performance once it was acquired. When affected significantly following administration of a drug, it underwent a reversible change, recovery eventually reaching or approximating predrug baseline levels. In both experiments changes were in the direction of slower response times.

2. The magnitudes of the decrements in performance were related to level of drug dose administered, the trend being for decrements to increase as dose level increased. Statistical tests at each drug's peak effect time showed the decrements to be very significantly different ($P < .01$) from the performance level of the corresponding placebo group.

3. The SAR illustrates a kind of limitation imposed on the study of time-response relations when appetitive drives provide the motivation required to generate the behavior under observation. Repeated measures of performance are required at intervals during the period when the absorption-metabolism of the drug is undergoing its major change. This usually means scheduling several test sessions within a period of less than 24 hours. This raises a technical problem since the drive is satiated at least to some extent during each test session and may not have returned to its standard controlled level before the next session. Under these circumstances, measures of behavior may reflect changes in motivation as well as drug effects. There are solutions to this problem, but they involve additional groups of subjects or additional test sessions, both of which increase significantly the level of effort required for completion of an experiment.

In the present experiments time-response relations could be described only in approximate terms. They did show, however, that peak effect times for both drugs were reached very rapidly and all behavioral effects had disappeared by the end of 24 hours after administration. At the highest Librium dose level the SAR recovered more rapidly than the SER.

4. Only a very tentative approximation could be made of the BD_{50} for Librium. The value, 7.7 mg/kg, differed in magnitude from the BD_{50} of 9.0 mg/kg for the SER. However, the tentative nature of the former provides no means of determining whether or not the difference is significant.

5. Librium had the effect of significantly increasing interindividual variability of SAR performance at the 20 minute and 24 hour test sessions following drug administration. This is another difference in that drug's effects on the SAR and the SER, the latter showing no significant effects of this kind.

6. Carry-over effects from one drug administration to another were significant ($P \leq .05$), as they were in the experiment on SER.

7. No significant effects were found of the particular order or sequence in which the various doses of Librium were administered.

8. The experiments provided information about the usefulness of the two research designs employed.

a. The presence of significant carry-over effects in the Librium experiment emphasizes, as did similar effects in the SER studies, one of the major problems in repeated observations using the same subjects. The randomized groups design removes any possibility of carry-over effects, since each subject receives only one drug administration. But this design raises very practical problems of the level of effort required to obtain as much information as the Latin square design provides at a lower cost; some kinds of information provided by the latter design cannot be obtained with the former, but this is information about interaction effects the presence of which complicates interpretation of results.

The analysis of the Librium data illustrates a possible way of combining the two types of analysis in one experiment. By replicating the Latin square design, the first drug series provides data from animals assigned at random to the several drug levels. Operationally this series is analogous to a randomized groups design. In the present Librium experiment five animals were assigned randomly to four dose levels, or groups, for the first test series; treatments of the animals in the second and subsequent test series were, of course, restricted by the requirements of the Latin square. When the results of the experiment were analyzed first as a Latin square design and later as a randomized groups design the dose-response relations in both cases were found to be significant, thus corroborating each other.

As was pointed out earlier when the general topic of research design was under discussion, the randomized groups design is less "iffy" than the Latin square design, where significant interactions may confound the results. However, when repeated measures on the same subjects is deemed to be a practical necessity, the Latin square design does provide information as to whether or not significant interactions have occurred.

b. Both research designs in the SAR experiments demonstrated one weakness which deserves attention. In neither case were the numbers of groups or dose levels sufficiently large to provide adequate data for computing BD_{50}'s. Had it been possible to follow up these pilot studies, fuller coverage would have been incorporated of the dose range between the point where no subjects were significantly affected by the drug and the point where 100 per cent were affected. This is a point to be considered during the preliminary studies leading to a final decision on the dose levels to be used in studying a new agent.

F. Fixed-ratio operant response (FR_{11})

The project's Report #1 described the desirability of including a wide range of behavior patterns in any behavioral screening program. The methodology of operant conditioning can be applied at many points in this range; the literature in psychopharmacology testifies to its wide application in research on drug-behavior interactions (e.g., Otis and Bosley, 1960). Sidman (1962) has described some of its advantages as follows:

> "It obtains its data from the individual; it uses the individual as his own control; it evaluates data in terms of its replicability and utility; it uses techniques that have species generality; it examines instances of variability."

The SER and SAR described above are examples of very simple operant responses. In the present project the basic methodology of operant conditioning was also employed in experiments designed to study more complex behavior patterns. Three of these were traditional lever-pressing responses performed under different reinforcement schedules. The present section describes two experiments in which the effects of Librium and pentobarbital on a fixed ratio operant response were studied; the next section of the report will describe studies involving variable interval reinforcement and the following section, a pilot study of differential reinforcement at low rates. Rats served as subjects in all experiments.

Research design. Both experiments employed Latin square designs. In neither case was there sufficient time to replicate the design.

The Librium experiment used a 4 x 4 Latin square with four animals, four drug series and four dose levels. Animals were assigned at random to the four rows of the design. The standard interval between drug administrations was one week. The dose levels were: 0, 4, 8 and 16 mg/kg.

The pentobarbital study followed a 3 x 3 Latin square design with three animals, three drug series and three dose levels. The doses used were: 0, 20 and 30 mg/kg. All other features of the research design were the same as those in the Librium experiment.

Subjects. Four subjects participated in the Librium series and three, in the pentobarbital series. All were male white rats of the Holtzman Albino stock. None had participated in previous experiments. In both studies they were randomly assigned to their respective treatment groups in accordance with the requirements of the research design. All were housed in separate cages during the experimental period and had water available *ad libitum*.

Test situation. In the FR situation the subject is required to respond a fixed number of times for each reinforcement. Attaining reinforcement is, therefore, "response contingent" in the sense that it depends upon the subject's own behavior. Characteristically, if the subject does not respond at close to his maximum rate, he does not respond at all. This is a kind of situation which occurs very frequently in the course of human behavior.

The general test situation has been so fully described so often in the literature that it is necessary only to call attention briefly to its main features from the point of view of the present experiments. The apparatus used was a standard Skinner-type of operant conditioning box constructed to Dr. C.B. Ferster's specifications (Ferster and Skinner, 1957) by technicians in his laboratory. A food pellet cup was placed immediately beneath a t-shaped lever extending from one wall of the box. An automatically programmed food magazine was located in a separate compartment. The programming rack containing relays, timers, power supplies, etc. necessary for controlling and programming the experimental procedure was kept in a separate room. The sound-deadening of the box and its location in a separate experimental room made it possible to control ambient noise, illumination and other distracting stimuli. A cumulative recorder kept a graphic record of all responses; they were also monitored on electric counters.

Procedure. The procedure consisted of four successive phases. The first phase involved establishing a controlled feeding schedule. The animals were first deprived of food and then brought to and maintained at 70 per cent of their *ad libitum* body weights. All were weighed and fed daily. When the FR training began, they were fed only after completion of the day's trials; the food available was determined for each animal by the amount necessary to maintain its body weight at the 70 per cent level.

The second phase centered upon shaping the lever-pressing response. The shaping process used procedures standard in operant conditioning methodology (Ferster and Skinner, 1957).

After the animals had learned to press the lever, they were put on an FR schedule of 11 responses per reinforcement, the ratio being chosen arbitrarily from several which had been used successfully in other studies. A day's test session consisted of 550 responses, the main measure of behavior being the time taken to complete this total. This procedure was followed in Phase 3 until each animal had established a stable response rate, the criterion being variability of not more than 10 per cent during a period of five successive days.

The drug phase began on the day after each animal reached this criterion. Drugs were administered in accordance with the research designs described earlier. All doses were administered orally by intubation. Administration was planned so that the first behavioral test session could begin one hour later in the Librium experiment and 20 minutes later in the pentobarbital study. Standard test sessions were then run at 24 hour intervals to a total of 144 hours, providing data for the study of time-response relations. All animals received their second doses on the seventh day and this weekly regimen continued until each animal had completed the number of drug series required.

Results. For purposes of analysis, raw time measures were converted to rate scores, the reciprocal of time. Each subject was used as his own control, changes in responses during the drug series being expressed as per cents of each subject's own predrug baseline performance.

1. Stability of baseline performance. The stability of the predrug baseline performance for each animal in the two FR_{11} experiments is shown in Table 38. The means, $\bar{x}$, and standard deviations, s, are based upon rates of responding during the last six test sessions preceding the first drug administration. The standard deviations indicate that the variability of responding during this period was small. Undoubtedly even this small intraindividual variability could have been brought closer to zero with further preliminary training. The practical question of relation between amount of extended effort and gain in the analysis of the results entered here and the criterion for stability described earlier was established.

To check on the maintenance of these predrug baseline levels during the drug series, each animal's rates were computed for the last two days, i.e., test sessions, of each week during the drug phase of the experiments. The results of experiments on other forms of behavior had shown recovery to be complete within 48 hours following administration of even the heaviest drug doses. It will be seen in Table 38 that, in all but one instance, performance on the last two days of each Librium series did not differ significantly from predrug baselines, i.e., was within ± 2 standard deviations of the predrug mean. The number of deviations was greater for the pentobarbital series.

Table 38

FR_{11}, Operant Response: Baseline Performance Levels

Animal	Predrug A[2] $\bar{x}$	Predrug A[2] s	Rate[1] Drug Series (Librium)[3] 1	2	3	4
1	0.40	0.03	0.42	0.43	0.45	0.36
2	0.61	0.02	0.58	0.62	0.57	0.54*
3	0.42	0.03	0.47	0.48	0.46	0.42
4	0.52	0.03	0.54	0.52	0.52	0.48

Animal	Predrug B[2] $\bar{x}$	Predrug B[2] s	Rate[1] Drug Series (Pentobarbital)[3] 1	2	3
1	0.40	0.02	0.40	0.31*	0.51*
2	0.54	0.03	0.45*	0.46*	0.53
3	0.49	0.03	0.53	0.47	0.47

1 1/time to make 550 responses.

2 Based upon the last six predrug test sessions.

3 Based upon the last two test sessions of each drug series.

* Beyond ± 2 standard deviations of predrug mean.

Figure 6. Fixed Ratio Operant Response (FR_{11}): Librium.

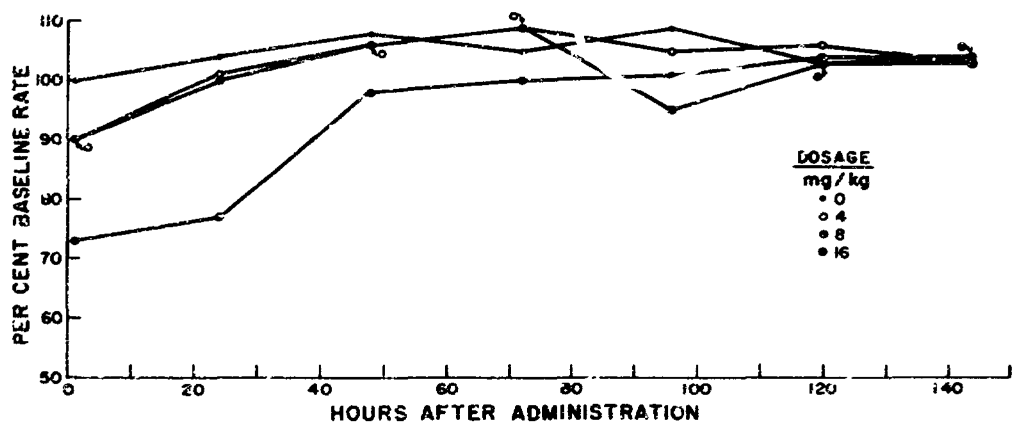
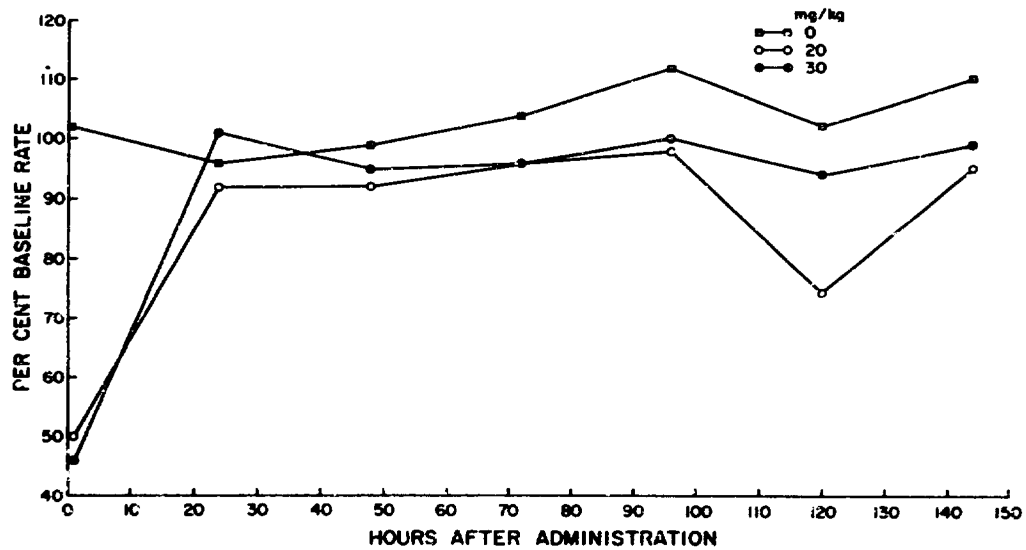


Figure 7. Fixed Ratio Operant Response (FR_{11}): Pentobarbital.



2. Dose-response relations. Tables 39 and 40 present the relative levels of performance at each test session after administration of the various doses of Librium and pentobarbital, respectively. Each number in Table 39 is the mean for the performance of four animals and in Table 40, the performance of three. Each was calculated from the per cent change from predrug baseline performance for each individual animal; in this way, the drug effects are evaluated in terms of each animal's own performance under non-drug conditions.

Inspection of Tables 39 and 40 suggest that both drugs had effects upon the FR_{11} response in the direction of an increase in time to perform the standard 550 responses as drug dose level increased. The significances of these drug effects at peak effect times were tested in the analyses of variance summarized in Tables 41 and 42; in the table drug effects are shown under "doses". The F-ratio for the Librium data was found to be significant at better than the .05 level of confidence. The corresponding F-ratio for the pentobarbital data was not significant, probably because of the small number of cases involved and, hence, the very restricted number of degrees of freedom available for the evaluation. Unfortunately, a replication of the design was not possible within the time limits of the project.

3. Time-response relations. Tables 39 and 40 provide information on time-response, as well as dose-response, relations during the six days following drug administration. In both instances the general trends are similar in shape to those characterizing the effects of the two drugs on other forms of behavior already described: decrements in performance developed very quickly after drug administration, probably reaching peak effects in one hour or less, and were followed by a more gradual recovery to approximately predrug baseline levels. The duration of the effect, defined in terms of hours after administration needed for significant differences from placebo baseline levels to disappear, was longer for Librium than for pentobarbital, the latter terminating in 24 hours or less and the former in 24 to 48 hours.

Table 39

Fixed Ratio Operant Response: Effects[1] of Librium on Performance Level

Time after administration (Hours)	Dose (mg/kg)			
	0	4	8	16
1	100	90	90	73
24	104	101	100	77
48	108	106	106	98
72	105	109	109	100
96	109	105	95	101
120	103	106	103	104
144	103	103	104	104

[1] Expressed as per cent of predrug baseline performance.

Table 40

Fixed Ratio Operant Response: Effects[1] of Pentobarbital on Performance Level

Time after administration (Hours)	Dose (mg/kg)		
	0	20	30
20 minutes	102	50	46
24	96	92	101
48	99	92	95
72	104	96	96
96	112	98	100
120	102	74	94
144	110	95	99

[1] Expressed as per cent of predrug baseline performance.

Table 41

FR_{11} Operant Response: Librium

Summary of Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
Doses	1,528.50	3	509.50	9.51	$< .025$
Weeks	3,690.50	3	1,230.17	22.96	$< .005$
Subjects	766.50	3	255.50	4.77	$< .05$
Error	321.50	6	53.58		
Total	6,307.00	15			

Table 42

FR_{11} Operant Response: Pentobarbital

Summary of Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
Doses	5,888.22	2	2,944.11	1.79	ns
Weeks	1,120.22	2	560.11	---	ns
Subjects	3,139.55	2	1,569.78	---	ns
Error	3,296.23	2	1,648.12		
Total	13,444.22	8			

4. Variability of performance. Data on interindividual variability of performance are presented in Tables 43 and 44 for the Librium and pentobarbital experiments, respectively. The usefulness of these data is questionable, since they are based upon such small samples of subjects. However, the analysis of variance for the Librium data, summarized in Table 41, indicated that significant interindividual differences did exist at the peak effect time, the "subjects" source of variance being associated with a "P" of about .05. When this fact is considered in examining Table 43, the standard deviations for the one-hour test session appear to have been increased under drug conditions, although there is no clear relation to dose levels. The data for the pentobarbital series are too scanty to warrant any speculation, despite what appears to be a very marked increase in variability with increasing dose level at the 20 minute test session.

5. Carry-over effects. The Latin square design used in the Librium study provided sufficient data for testing the possible significance of carry-over effects. It will be seen from Table 41 that the F-ratio for "weeks" was highly significant. This finding fits well into the general pattern for Librium which also chowed significant carry-over effects in experiments on the SER and SAR. Although any conclusion from the FR_{11} pentobarbital series must remain tentative until more subjects have been studied, it is of interest to note the apparent lack of carry-over effects in both the FR_{11} and SER experiments. Data on the former are summarized in Table 42.

Summary. The results of these two pilot studies may be summarized briefly. Both experiments used rats as subjects, one in studying effects of Librium on the FR operant response and the other, effects of pentobarbital. The drugs were administered by intubation: Librium in doses of 0, 4, 8 and 16 mg/kg and pentobarbital in doses of 0, 20 and 30 mg/kg. Both studies employed Latin square designs; time limits of the project did not permit replications of either design.

Table 43

Fixed Ratio Operant Response: Effects of Librium on Interindividual Variability of Performance[1]

Time after administration (Hours)	Dose (mg/kg)			
	0	4	8	16
1	8.06	26.08	18.28	22.65
24	8.94	8.19	3.46	5.74
48	8.43	3.32	3.00	11.27
72	9.80	10.68	9.38	6.40
96	9.43	10.10	25.55	11.70
120	15.78	14.25	9.00	4.47
144	7.14	5.66	6.32	5.92

[1] Expressed as standard deviations, s.

Table 44

Fixed Ratio Operant Response: Effects of Pentobarbital on Interindividual Variability of Performance[1]

Time after administration (Hours)	Dose (mg/kg)		
	0	20	30
20 minutes	4.58	21.21	57.50
24	7.21	3.61	10.54
48	5.20	11.58	6.40
72	4.00	10.68	8.49
96	18.00	8.00	3.00
120	33.31	17.09	6.93
144	9.17	2.45	44.46

[1] Expressed as standard deviations, s.

1. The FR situation provides an opportunity to study effects of drugs on behavior in which reinforcement is "response contingent", i.e., attaining reinforcement depends upon the subject's own behavior. The standard procedure for establishing the response provided very stable predrug baselines of performance once the response was acquired.

2. One of the effects of both drugs appeared to be evidenced in an increase in time to perform the standard number of responses as drug dose level increased. Analyses of variance showed the effect to be significant in the Librium data and not in the pentobarbital series, probably because of the small sample in the latter study.

3. Time-response relations took the familiar form in which decrements in performance developed rapidly, probably reaching peak effect times in one hour or less, and were followed by more gradual recivery. Recovery required a longer time, 24 to 48 hours, after administration of Librium than after pentobarbital, within 24 hours.

4. The usefullness of data about interindividual variability of performance is questionable because of the small sample of subjects studied. Analysis of variance of the Librium data showed significant "subjects" variance at the peak effect time, which may be accounted for in terms of an increase in variability under the drug conditions.

5. Significant carry-over effects were found in the Librium series, corresponding to similar effects of that drug on the SER and SAR.

6. The results of the two experiments demonstrated that studies of drug effects using unreplicated Latin square designs as limited as 4 x 4 can provide statistically

significant results. In experiments reported so far such designs have ranged from 3 x 3 to 5 x 5; replications have ranged from none to five. Although the more limited designs may produce some useful information, it is clear that they do not provide sufficient data for such analyses as log-probit determinations of ED_{50}'s.

G. Variable interval operant response (VI) and conditioned emotional response (CER).

The fact that normal behavior patterns may be disrupted or interferred with by emotional disturbances is a common observation in everyday life. The enhancement of such disturbances would be a useful property for an incapacitating chemical agent.

During the past several years, considerable attention has been given to the development of experimental techniques by which the effects of a "controlled" emotional response on some other ongoing behavior may be studied. One of the most frequently used techniques involves superimposing a "conditioned emotional response" (CER) upon some other form of behavior, e.g., lever-pressing behavior. Using lever-pressing as an example, the technique may be described as involving the following successive phases. The lever-pressing response is reinforced on a variable-internal schedule until response rates have stabilized. Next a CER is superimposed upon the lever-pressing behavior by presenting an auditory stimulus at regular intervals and for fixed durations during a lever-pressing session, the stimulus terminating with a brief, painful electric shock to the animal's feet. After several sessions with shock following every presentation of the auditory stimulus, the schedule is changed so that shock occurs only occasionally after termination of the stimulus. The CER resulting from this training is characterized by suppression of lever-pressing, crouching, defecation, and immobility. With sufficient training, the ratio of number of lever responses during CER periods to the number of lever responses when the CER is not superimposed remains stable. Studies have shown differential effects of different chemical agents upon the suppression of a normal behavior pattern by the CER (Hunt, 1960). The CER was included as one of the behavior patterns to be studied in the present project.

Since the procedure for measuring the conditioned suppression involved establishing and recording a lever-pressing response reinforced on a variable-interval schedule, each experiment could provide information about the effects of a drug on the VI operant response as well as on the CER. Sidman (1960) has pointed out that, if the VI schedule is properly constructed, subjects will respond at steady rates and performance will be sensitive to many experimental manipulations, e.g., drug administration.

Research design. Two pilot studies were conducted, one involving the administration of Librium and the other, pentobarbital. Both used 3 x 3 Latin square research designs, with no replications. Each study required three animals, three drug series and three dose levels. Rats served as subjects, being assigned at random to the three rows of the design. The standard intertreatment interval was one week; one test session was given each day.

Test situation. The general requirements for the test situation were described in the introduction to this section. Both the VI operant response and the CER were established and measured during drug treatments in the same Skinner-type box used in the FR_{11} experiments. The only additions to the apparatus were those needed for establishment of the CER, i.e., a controlled shock to the grid floor of the box and a controlled clicking sound. The shock was produced by a Siddons shock generator calibrated in the low milliampere range which passed current through a standard scrambler to the grid floor. The auditory stimulus was fed into a loudspeaker, located in the box, by a transistorized square wave generator.

Subjects. Six male white rats of the Holtzman Albino stock served as subjects, three in each experiment. They had not participated in any previous studies. All were housed in individual cages during their participation in the experiments. They were watered *ad libitum* in their home cages.

Procedure. Since food-hunger served as the form of motivation in both studies, Phase 1 of the procedure was devoted to establishing a controlled feeding schedule. The animals were first deprived of food and then brought to and maintained at 70 per cent of their *ad libitum* body weight. All were weighed daily and were fed in their home cages after completion of a day's test session. The amount of food made available was that necessary to maintain body weight at the 70 per cent level.

Phase 2 consisted of shaping the lever-pressing response and then training each animal on a one-minute VI schedule. Fifty reinforcements were given during a day's test session. This training continued until a steady response rate had been established to the criterion of 10 per cent or less variability for a period of five successive days.

Training of the CER began the day after this criterion was reached. The existing VI, schedule was divided into 10-minute units and the clicker-shock combination introduced during the last three minutes of each unit, so that the clicker sounded for the full three minutes and was terminated by a half-second of shock. During the first CER session a shock level of 0.2 m.a. was used. Each succeeding day the level was raised by 0.2 m.a. until a 1.0 m.a. level was reached at the end of five days. This shock level was maintained throughout the remainder of the experiment.

The final phase of each study involved administration of the various drug doses in accordance with the requirements of the research design. The dose levels for Librium were 0, 8 and 16 mg/kg abd for pentobarbital, 0, 10 and 20 mg/kg. All doses were administered orally by intubation. The Librium doses were given one hour before the first test session and the pentobarbital doses, 20 minutes in advance.

Results. The standard measure for the VI_1 response was the rate of responding during the seven-minute periods when no clicker was sounding. The measure of CER was expressed as a ratio between rates of responding during the clicker and the non-clicker periods.

1. Stability of baseline performance. Table 45 contains the means, $\bar{x}$, and standard deviations, s, of predrug VI_1 response levels for each of the three subjects participating in the two studies; Table 46 presents analogous data for the CER. Two features of the information in the tables deserve particular attention. One is the wide inter-individual differences which are clearly apparent in both the VI_1 and CER measures. Other experimenters have reported such interindividual differences in response rates, but have found the rate to be steady during a test session and to be "relatively consistent during many successive experimental periods (Sidman, 1962)". The standard deviations, s, in Tables 45 and 46 indicate that this "relative" consistency characterized the predrug performance rates of some but not all of the present subjects: rates were steady during a test session, but, in some cases, tended to vary from session to session. It appears that the criterion for stable predrug baseline performance should have been a more stringent one.

The second feature deserving some special attention from the methodological point of view is illustrated by animal no. 1 of the Librium series in Table 46. This animal's mean predrug CER of 167 indicates that its rate of responding during the clicker-shock period was higher than it was during the period when the clicker was not sounding, i.e., the opposite of conditioned suppression occurred. This particular animal went through his life in the present study in this consistently "euphoric" manner. This and other instances in which it proved difficult to establish a stable CER appear to indicate that the response, at least during its acquisition, is sensitive to several major parameters of the test situation and that an experimenter may have to be "artful" in finding the best values for each parameter when conditioning different animals.

Table 45

VI_1 Operant Response: Predrug Baseline Performance Levels

A. Librium

Animal	$\bar{x}$	s
1	5.0	0.48
2	21.0	2.32
3	17.7	2.58

B. Pentobarbital

Animal	$\bar{x}$	s
1	15.2	2.07
2	4.7	1.24
3	9.4	2.98

Table 46

Conditioned Emotional Response: Predrug Baseline Performance Levels

A. Librium

Animal	$\bar{x}$	s
1	167	24.82
2	55	14.07
3	36	7.75

B. Pentobarbital

Animal	$\bar{x}$	s
1	56	14.53
2	46	16.58
3	13	8.43

2. Drug effects. Gross inspection of the data obtained during the drug series failed to show any such clearly discernible drug effects as had been readily perceived with other forms of behavior. Despite severe limitations imposed by the very restricted degrees of freedom, i.e., d.f. = 2, available with the present research design and sample size, analyses of variances were carried out for the VI_1 and CER data. The results of these analyses are summarised in Tables 47 and 48. There were no significant F-ratios in either analysis; therefore, the data were not examined further.

Summary. Both the VI and CER are behavior patterns which would appear a priori to be prime candidates for inclusion in a behavioral screening battery. The present pilot studies served a useful purpose, despite their failure to turn up any firm results. Other experimenters have also reported problems in establishing stable CER's in rats; such appears to depend upon specific details of technique, e.g., initial shock intensity, criterion for stability of CER, etc. Had time permitted the conduct of another CER study, the procedures used in the present experiments would have been modified in some details and the criterion for a stable pre-drug baseline would have been made more rigorous.

Table 47

VI_1 Operant Response

Summary of Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
		A. Librium			
Dose	12,014.22	2	6,007.11	—	ns
Subjects	45,706.89	2	22,853.45	2.68	ns
Weeks	22,221.55	2	11,110.78	1.30	ns
Error	17,061.56	2	8,530.78		
Total	97,024.22	8			
		B. Pentobarbital			
Dose	1,432.89	2	716.45	2.02	ns
Subjects	80.89	2	40.45	—	ns
Weeks	1,536.23	2	768.12	2.17	ns
Error	709.55	2	354.78		
Total	3,759.56	8			

Table 48

Conditioned Emotional Response

Summary of Analysis of Variance

Source of Variation	Sum of Squares	d.f.	Mean Square	F	P
		A. Librium			
Dose	770.89	2	385.45	3.59	ns
Subjects	1,638.22	2	819.11	7.62	ns
Weeks	3,968.22	2	1,984.11	18.47	ns
Error	214.89	2	107.45		
Total	6,592.22	8			
		B. Pentobarbital			
Dose	4,730.89	2	2,365.45	3.82	ns
Subjects	4,842.89	2	2,421.45	3.91	ns
Weeks	6,500.22	2	3,250.11	5.25	ns
Error	1,238.22	2	619.11		
Total	17,312.22	8			

H.. Timing behavior (DRL)

An individual's control over his own behavior is often based upon his ability to respond at proper time intervals, e.g., he must be able to delay his response for a specific period of time if he is to perform adequately. Studies of delayed responses have received particular attention from comparative psychologists as a means of investigating experimentally the "higher mental processes (Stone, 1951)." The assumption is made that delayed responses involve the use of some representational factor, that temporal delays require some symbolic process since no immediate environmental cues are available upon which to base adequate responses. The possibility that timing behavior may have a biochemical basis has been suggested by Hoagland (1934 & 1935): the results of several experiments involving temperature analysis, via the Arrhenius equation, of estimations of time durations "...definitely imply the existence of a unitary chemical process serving as a basis for the subjective time scale, a process probably irreversible in nature and perhaps catalyzed in a specific way...(Hoagland, 1934)." Evidence has been presented that changes in time perception of human subjects may be induced by drugs (e.g., Steinberg, 1955). Because of the wide involvement of timing behavior in complex human activities, the possibility of "incapacitating" such activities by the use of chemical agents deserves investigation.

The present project included a pilot study of timing behavior, using rats as subjects. The behavior pattern involved in the study required the subjects to space their responses at least 18 seconds apart in order to receive reinforcement. Since responses that occurred at a rate less than one per 18 seconds were not reinforced, the schedule is referred to as the differential reinforcement of low response rates abbreviated "DRL" (Ferster and Skinner, 1959; Anger, 1956; Sidman, 1955). The purpose of the pilot study was to obtain information upon which to base the precise conditions and procedure for a DRL measure which might be included in the final behavioral test battery.

Research design. If administration of the drug produced changes in the DRL response, measures of behavior during drug test sessions would be expected to differ significantly from measures during non-drug sessions. The measure of behavior used was the time between responses or the "interresponse time," abbreviated "IRT." The research was designed, first, to provide means and standard deviations of the distribution of IRT's for performance under non-drug conditions and, second, to obtain IRT's following administration of the drug or placebo. Deviations of the latter which were two standard deviations or more from the mean of the non-drug distribution could then be considered "significant" in terms of the usual statistical conventions.

Test situation. The apparatus used to study DRL responses was a Skinner-type box, 5 5/8 inches wide by 10 inches long by 6 3/4 inches high. The walls were made of aluminum and the ceiling of clear plastic; the floor consisted of fine aluminum tubes. The manipulandum, a bar, was mounted on one wall and required 10 grams pressure to close the response circuit. The box was housed in a light and sound attenuated, ventilated cabinet. All contingencies were programmed by automatic electro-mechanical equipment. Water was constantly available in both the living cages and the experimental chamber.

During each test session an animal continued performing until approximately 100 reinforcements were obtained. Reinforcement consisted of a 97 mg. Noyes food pellet. The reinforcement schedule was so programmed that the subjects had to space their responses at an IRT of 18 seconds in order to receive a pellet. Reinforcement was withheld if a response occurred in less than 18 seconds following the previous response; there was no penalty for responding at longer IRT's. Responses during each test session were recorded graphically on a cumulative recorder.

Subjects. Since this was a pilot study to examine the DRL situation as a candidate for inclusion in a final behavioral test battery, it was decided to concentrate upon the intensive observation of only two subjects, but to do this over an extended period of several months. Two male white rats of the Holtzman Albino Stock served as subjects. They had not participated in any previous experiments. When not in the experimental apparatus, each was housed in a separate home cage where water was available *ad libitum*.

Procedure. The procedure consisted of three basic phases. Phase 1 involved the establishment of a controlled feeding schedule. The animals were first deprived of food and then brought to and maintained at a 70 per cent of their *ad libitum* body weight.

After the feeding schedule had been established, the animals were trained to displace the response bar for the food reinforcement. At this point the DRL schedule was introduced. Training on this schedule continued daily, each subject being run until 100 reinforcements were obtained. The only deviation from this procedure occurred on days when drug or placebo doses were administered; these special treatments constituted the third phase of the study.

Both drug and placebo doses were administered orally by intubation in the manner standard for all studies in the project. The chemical characteristics of the placebo were described earlier; the Librium doses were given as fresh aqueous solutions. In order to explore the period of maximum influence of the drug as defined by its effects on other measures of behavior already described, test sessions began at 1, 3, or 5 hours following treatment, as indicated in the description of results to follow. The drug series were introduced at irregular intervals, a minimum of 20 days separating treatments. In a final drug series the 16 mg/kg dose was given intraperitoneally in order to observe effects of a second route of administration.

Results. The experimental results consisted of distributions of IRTs with modal points in the region of 18 seconds. For convenience, data forming the various distributions were analyzed in step intervals of three seconds each from zero time delay to a final interval of 39 seconds and above. As will be discussed in more detail below, the distributions under non-drug conditions approximated the form of the normal curve. Tests for drug and placebo effects involved evidence that the distributions under these two conditions differed significantly from the non-drug or baseline distributions.

1. *Characteristics of baseline (non-drug) performance*. The advantages of using each subject as his own control were discussed earlier. The comparisons involved require information about the level of non-drug, baseline performance. Table 49 summarizes the frequency distribution of interresponse times based upon measures taken during the 25 test sessions immediately preceding the first drug series. The second column of the table presents the mean per cent of IRTs

falling at each step interval during the 25 trials and the third column shows the variability of the per cents in terms of standard deviations. It is clear that the variabilities are relatively large, indicating that considerable change in IRTs occurred from day to day: further study of factors affecting the stability of the predrug baseline would be in order before including this measure of behavior in a final test battery. Examination of the second column shows that 21 per cent of the IRTs occurred below the point, 18 seconds, when reinforcement began. At 18 seconds the frequency of IRTs increased substantially, the modal point for the overall distribution being at the 21-23 seconds interval, and the distribution then tailed off rapidly. The mean response rate during the 25 trials was 2.8 ± 0.2 responses per minute. When plotted the distribution is seen to be very symmetrical, resembling a normal curve except for excessive responses at the 0-2 seconds interval. The latter feature of the distribution is not unusual for this kind of response; it is referred to as "bursting" and is commonly found to comprise as much as 30 per cent of the total responses in the DRL situation. In the present study bursting never exceeded eight per cent, indicating an unusually high degree of control over the DRL performance.

Distributions of baseline performance between the various drug and placebo series are summarized in Table 50. The distributions are based upon measures of performance from 75 non-drug test sessions. Separate distributions are reported for each of the two subjects since these were the baseline data with which performance during the drug and placebo series were compared. The distributions are very similar to the predrug distribution described above. They are very symmetrical in shape, with modal points at the 21-23 and 18-20 second intervals for subjects 1 and 2, respectively. Both show some bursting at the shortest IRT interval. The response rates for the two subjects were 2.8 and 2.9 responses per minute as compared to a response rate of 2.8 for the predrug test sessions. This close similarity between predrug and non-drug baseline performance indicates that, despite the day-to-day variability noted earlier, a basic level of stability in performance was achieved by the preliminary training procedure, a level to which performance returned after drug treatments.

Table 49

DRL Response: Distribution of IUTs

During Predrug Test Sessions[1]

Interresponse Times (Seconds)	Mean Per Cent	S
0- 2	6.3	2.9
3- 5	0.6	0.8
6- 8	0.8	1.2
9-11	1.2	1.3
12-14	3.5	2.1
15-17	8.7	3.4
18-20	22.2	6.4
21-23	25.2	4.3
24-26	16.7	4.7
27-29	7.2	3.2
30-32	3.4	2.5
33-35	1.7	1.7
36-38	1.0	1.2
> 39	1.1	1.9
Response Rate[2]	2.8	0.2

[1] Based upon measures taken during 25 test sessions.

[2] Responses per minute

Table 50

DRL Response: Distributions of baseline (non-drug) IRTs[1]

Interresponse Time (Sec.)	Subject 1 Mean Per Cent	S	Subject 2 Mean Per Cent	S
0- 2	4.4	2.2	6.8	2.5
3- 5	0.5	0.9	0.5	0.6
6- 8	0.6	0.8	0.7	0.8
9-11	2.0	2.1	1.9	1.4
12-14	6.0	2.9	5.6	2.7
15-17	12.9	3.9	13.5	4.3
18-20	20.5	7.2	28.5	7.6
21-23	21.5	5.3	23.5	5.3
24-26	14.8	4.1	10.7	5.3
27-29	8.3	4.2	4.7	2.9
30-32	4.8	3.8	1.7	1.7
33-35	2.3	2.4	0.8	1.0
36-38	1.0	1.2	0.4	0.6
39 +	0.9	1.3	0.5	0.8
Response Rates[2]	2.8	0.3	2.9	0.4

[1] Based upon measures taken during 75 non-drug test sessions.

[2] Responses per minute.

2. Placebo effect. The research was designed to provide information about any effect which might have resulted from the intubation procedure itself regardless of the chemical agent administered. Distributions of IRTs following placebo administration are summarized in Table 51. Measures were taken of performance during test sessions which began one, three and five hours after administration of placebo doses. All four distributions in Table 51 are very similar to the corresponding distributions for baseline performance as summarized in Table 50. In fact, all values for subject 1 fall within ± two standard deviations of the corresponding values for non-drug baseline performance. For subject 2 three values show significant changes during the one hour test session, while only one value just reaches the criterion for significance at the three hour session. Where significant effects do appear, they are in the direction of higher per cents of longer IRTs and are most pronounced at the 27-29 seconds interval. Administration of the placebo produced no significant changes in response rates.

3. Drug effects. Very consistent effects on performance were found following oral administration of Librium in doses of 16 mg/kg. Examination of Table 52, which summarizes data from both subjects for test sessions one and three hours after drug administration, shows three main features of the effects: first, they appear as increases in per cents of longer IRTs; second, the effects are pronounced during the three-hour test sessions and almost completely absent during the one-hour sessions; third, the response rates, which constitute the most frequently used measure of operant behavior, show a tendency to decrease, the value for subject 1 at three hours after drug administration being significantly lower than its corresponding baseline value.

Similar results were obtained when intraperitoneal injection was used as the route of administration, the Librium dose remaining at 16 mg/kg. Table 53 shows the distributions of IRTs for test sessions begun 15 and 30 minutes after injection. Again the predominant feature of the distributions is a shift toward higher per cents of longer IRTs, although, in contrast with effects following oral administration, increases also appeared at IRT intervals in the region of three to eight seconds. In both instances response rates decreased significantly. The most striking differences between the data in Tables 53 and 52 appear in terms of the much shorter latency in the production of significant effects following intraperitoneal than following oral administration of the drug.

Figure 3. Log-Probit of Dose-Response Curves for Acute Oral Administration of Librium in the Rat.

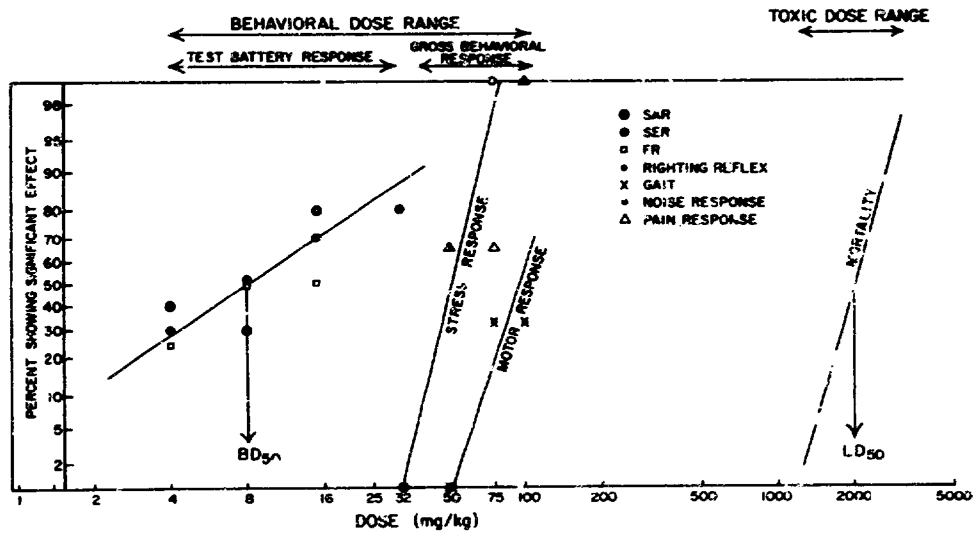


Table 51

DRL Response: Placebo Effects

Interresponse Time (Sec.)	Per Cent of Responses Subject 1 1 hr.	Subject 1 5 hr.	Subject 2 1 hr.	Subject 2 3 hr.
0- 2	4.7	3.7	1.8	7.1
3- 5	0.0	0.0	0.0	0.0
6- 8	0.0	0.0	0.0	0.8
9-11	3.4	1.2	0.0	0.0
12-14	5.4	7.6	2.7	2.4
15-17	12.2	8.7	7.1	8.7
18-20	24.3	26.5	19.4	23.0
21-23	23.6	26.1	26.5	22.2
24-26	12.2	10.6	15.9	18.2
27-29	9.4	11.2	16.8*	8.7
30-32	2.7	3.7	3.5	4.8
33-35	1.4	0.0	4.4*	1.6
36-38	0.7	0.6	1.8*	1.6*
39 +	0.0	0.0	0.0	0.8
Response Rate	2.8	2.8	2.5	2.7

* More than + 2 standard deviations from the baseline mean.

Table 52

DRL Response: Drug Effects[1] (Oral Administration)

Interresponse Time (Sec.)	Per Cent of Responses: Subject 1		Subject 2	
	1 hr.	3 hr.	1 hr.	3 hr.
0- 2	4.7	1.7	4.2	4.8
3- 5	0.8	0.0	0.0	0.0
6- 8	0.8	1.7	0.0	0.7
9-11	3.1	0.0	3.5	2.7
12-14	3.9	4.3	7.7	7.5
15-17	9.3	7.7	13.3	10.3
18-20	14.0	6.0**	30.8	12.3**
21-23	16.3	9.4**	20.3	11.6**
24-26	12.4	15.4	13.3	16.4
27-29	17.8*	18.0*	4.9	14.4*
30-32	11.6	11.1	0.7	13.7*
33-35	2.3	8.6*	0.7	3.4*
36-38	1.6	7.7*	0.7	2.1*
39 +	0.8	8.6*	0.0	0.0
Response Rate[2]	2.5	2.1**	2.9	2.5

[1] Dose: 16 mg/kg body weight.

* More than + 2 standard deviations from the baseline mean.

** More than - 2 standard deviations from the baseline mean.

[2] Responses per minute.

Table 53

DRL Response: Drug Effects[1] (IP Administration)

Interresponse Time (Sec.)	Per Cent of Responses Subject 1 15 min.	Subject 2 30 min.
0- 2	6.7	3.1
3- 5	2.5*	6.2*
6- 8	0.0	4.1*
9-11	0.8	1.0
12-14	1.7	1.0
15-17	5.0**	4.1**
18-20	3.3**	6.2**
21-23	15.0	4.1**
24-26	18.3	6.2
27-29	11.7	8.2
30-32	11.7	9.3*
33-35	5.8	8.2*
36-38	9.2*	5.2*
39 +	8.3*	31.9*
Response Rate[2]	2.2**	1.1**

[1] Dose: 16 mg/kg body weight.

* More than + 2 standard deviations from the baseline mean.

** More than - 2 standard deviations from the baseline mean.

[2] Responses per minute.

Figure 9. Absorption Curves for Librium and its Acid Hydrolysate.

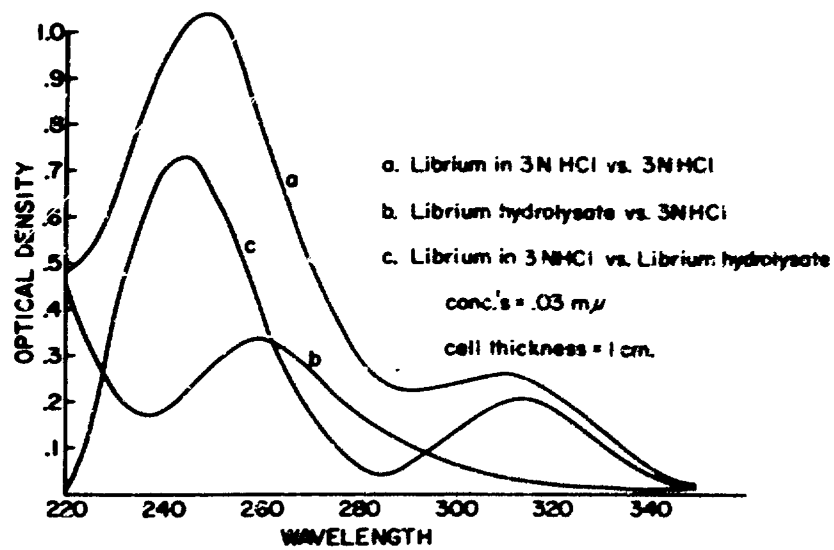
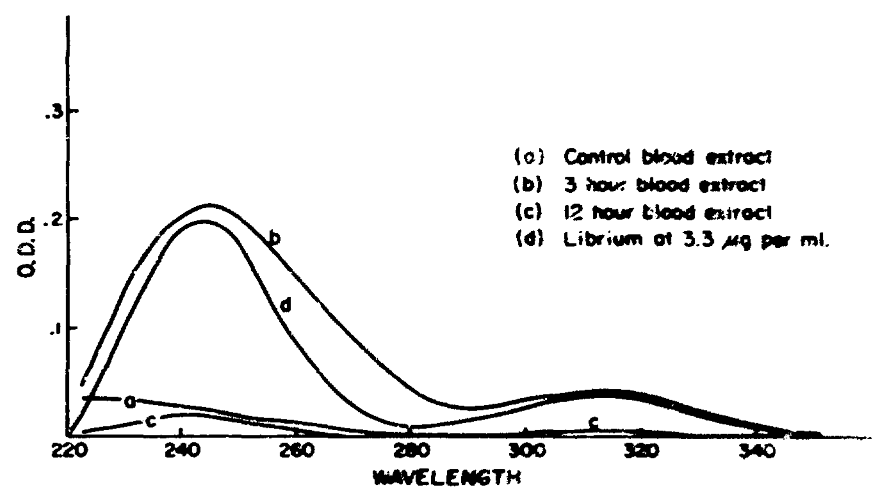


Figure 10. Absorption Curves for Plasma Extracts from Librium-Treated Rats.



Summary. This pilot experiment was planned as a preliminary study of timing behavior, a form of behavior which is important in many types of repetitive or sequential tasks where adequate performance depends upon responding at proper time intervals. The behavior pattern involved in the present study required the subjects to space their responses at least 18 seconds apart in order to receive reinforcement. Intensive observations were made of the behavior of two subjects over an extended period of several months. The chemical agent used was Librium, administered both orally and intraperitoneally. Rats served as subjects. The results are summarized below.

1. The results confirmed those obtained by other experimenters who have studied this kind of timing behavior: it is a difficult performance pattern for the rat to learn. However, as the experimental data show, sufficiently stable performance was established to reflect sensitivity to drug effects. The measure of behavior, i.e., time between responses or IRT, provided distributions of responses during each test session. Such distributions were obtained during test sessions under the following conditions: predrug baseline performance, non-drug baseline performance between drug and placebo series, performance following placebo administration, and performance following oral and intraperitoneal administration of Librium at a dose level of 16 mg/kg.

2. The measures of baseline performance under both predrug and non-drug conditions were very symmetrically distributed around modal points between 18 and 23 seconds interresponse time. An obvious deviation from the normal curve appeared at the shortest step interval, 0-2 seconds, where excessive numbers of responses occurred. However, this feature of DRL behavior, known as "bursting", appeared less frequently than usually reported by other experimenters, indicating that the present procedures had resulted in an unusually high degree of control over the DRL performance. Predrug baseline levels of performance were re-established following administrations of drug or placebo, both the distributions and response rates for trials between drug and placebo series being very similar to those obtained during predrug tests.

3. Response rate, the most frequently used measure of operant behavior, was not affected by administration of the

placebo. It was, however, sensitive to the drug as evidenced by decreases in responses per minute following both oral and intraperitoneal administration.

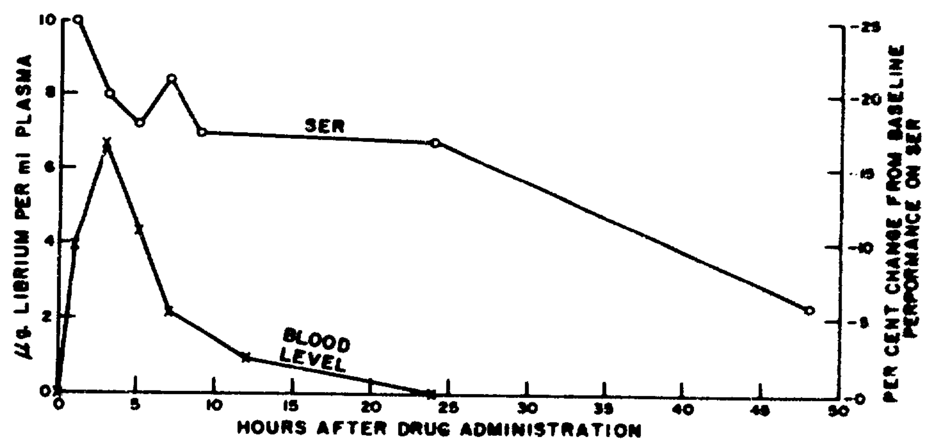
4. Examination of IRT distributions shows that the decreases in response rates following drug administration were associated with shifts in the distributions toward larger per cents of longer IRTs. Subject 2 did show a placebo effect in this same direction, but the effect was much less pronounced than that produced by the drug. The general picture was one in which the effects of the drug favored interresponse times of the kind being reinforced, i.e., those 18 seconds or longer.

5. The time-response characteristics of the drug effects had one feature of particular interest. Peak effect times for other behavior patterns described earlier in this report were reached <u>within</u> one hour after oral administration of Librium at the dose level used in the present pilot study. In contrast, the DRL response showed no pronounced effects until some time <u>after</u> one hour. This contrast illustrates a second way in which drugs may produce differential effects on different behavior patterns, i.e., differences in time-response characteristics. The first was demonstrated in experiments described earlier, appearing as differences in dose-response characteristics.

As would be expected, the intraperitoneal route of administration cut this peak time considerably, significant effects appearing after 15 minutes and becoming very pronounced after 30 minutes.

6. The results of the present pilot study would [illegible] encourage further examination of the DRL response as a candidate for inclusion in a final behavioral test battery. Changes in training procedure aimed at decreasing day-to-day variability in IRTs deserve attention; further data on dose-response and time-response relations should be obtained; and comparisons should be made between effects of several chemical agents in order to determine whether this behavior pattern is differentially sensitive to agents with different chemomorphologies.

Figure 11. Time-Response Curves for Drug Blood Level and SER Effects: Librium.



I. Straightaway approach response to differential reinforcement (SAR-D)

In the continual process of adjusting to their environments living organisms are often exposed to situations in which they are prevented from or delayed in reaching their goals by contingencies beyond their own control. Under such circumstances behavior may become disorganized and "displacement activities" appear. In both instances the effects are "incapacitating". A very extensive literature has developed around experimental studies of "frustration" and "conflict" in both human and infrahuman animals since Pavlov's first research on "experimental neurosis" (see Russell, 1950 and 1953).

The pilot study described briefly in this section of the present report was a first step toward devising a standardized situation in which the effects on behavior of exposure to short periods of "frustration" in which reward was delayed by decreasing the ratio of reinforcement could be compared with the control performance of the same subject during the same experimental session. The general procedure made use of two schedules of reinforcement. In one, each trial ended with reward; in the other, a trial might or might not end with reward. The same operant response, the straightaway approach response described in section IV E above, was used with both schedules. The only differential environmental cues were the visual stimulus patterns of the two alleys in which responses under the two schedules were made.

Research design. As indicated above, each animal performed under both reinforcement schedules during each experimental session. As a means of controlling for possible effects of the two different alleys used, n = 16 subjects were assigned at random to k = 2 groups. Animals in one group received 100 per cent reinforcement at the completion of each trial in one alley and 50 per cent reinforcement in the other; the relations between alleys and reinforcement schedules were reversed for the other group. Effects of Librium on performance under the two schedules of reinforcement were studied after baseline performance levels had been established.

Subjects. Sixteen male white rats of the Holtzman Albino stock served as subjects. They had not participated in any previous experiments. When not in the experimental apparatus each was housed in a separate home cage, where food was available *ad libitum*.

Test situation. The apparatus consisted of an enclosed double straightaway, one alley of which was painted black and the other, black with white stripes. Each was composed of a starting box, a straightaway segment, and a goal box. A subject's movements through the apparatus could be monitored by three photoelectric cells: one at the exit of the starting box, the second at the opposite end of the straightaway segment and the third inside the goal box one inch in front of the water incentive. In the present study the straightaway approach response, SAR-D, was measured in terms of the time between a subject's exit from the starting box and his entrance into the goal box. Interruption of the first photocell beam tripped a timer, which was stopped when the subject passed through the second photocell beam. The measure of performance was time taken to traverse the straightaway segment; this measure was transformed to log units in analyzing the results of the study.

Procedure. The procedure consisted of three major phases.

During phase 1 a standard 23-hour water deprivation schedule was established.

During phase 2 all animals were given 19 daily blocks of eight trials, four in each alley of the apparatus. Each trial began by placing the animal in one of the starting boxes and the starting-box gate raised. The animal left the box and interrupted the first photocell beam thus activating the timer and the gate was lowered behind him. The goal-box gate was lowered once the animal had entered that box and had interrupted the photocell beam stopping the timer. On rewarded trials the animal was allowed to drink for five seconds; on non-rewarded trials, he was detained in the goal box for the same period of time. The order in which trials were run in the two alleys was randomized, with the restriction that, during each daily session, four trials had to be in the 50 per cent and four in the 100 per cent rewarded alleys. The intertrial interval was three minutes. The incentive consisted of 20 per cent, by volume, sugar solution.

Phase 3, the drug phase, began after completion of the acquisition trials. The animals were given three additional daily blocks of trials using the same procedure as in phase 2. Before the first of these sessions the subjects were divided into four groups of equal size, a control and three drug groups. Drug doses were administered by intubation one hour before the first of the three daily sessions; the second and third sessions were run 24 and 48 hours after administration. The control group received the standard placebo for Librium as described earlier in this report; the three drug groups received 4, 8 and 16 mg/kg of Librium, respectively.

Results. Table 54 summarizes the mean time scores for all animals during the acquisition trials of phase 2; times are given in log units. Table 55 gives the time scores for daily blocks of trials during the drug phase of the study. For present purposes the results may best be presented under three major headings.

1. Trends during acquisition trials. Examination of Table 54 shows that performance at the start of the SAR-D trials was at almost identically the same level under both reinforcement conditions. From day 2 to day 5 speed of running was faster during 100 per cent than during 50 per cent reinforced trials, a t-test showing the difference to be significant at the $p < .05$ level. There followed three days during which the difference disappeared. As trials continued a significant difference again developed, $p < .01$, but in the opposite direction to that of days 2-5, i.e., from day 9 to 13, the animals ran faster under conditions of 50 per cent reinforcement. Although this oscillation decreased greatly in magnitude beginning with day 14, differences between measures of performance under the two conditions of reinforcement becoming very small, it did continue to be evidenced throughout the remainder of phase 2 and also in the behavior of the control group in phase 3.

2. Displacement activity. During the period when the oscillation of performance between the two conditions of reinforcement showed its greatest magnitude, interesting displacement activities appeared. These were most obvious during days 9 to 13 under conditions of 50 per cent reinforcement and were evidenced as marked increases in defecation, urination, squeaking and rapid circling movements. Some of the animals chewed at the apparatus and attempted to bite the experimenter while being handled during intertrial periods. These activities were not noticeable during trials before or after this five-day period.

Table 54

Straightaway Approach Response to Differential Reinforcement: Time Scores During Acquisition

Daily Trial Block[1]	Mean Log Time 50% Reinforcement	Mean Log Time 100% Reinforcement
1	-0.59	-0.60
2	-0.34	-0.23
3	-0.21	-0.18
4	-0.04	+0.04
5	-0.04	+0.05
6	+0.11	+0.12
7	+0.16	+0.16
8	+0.20	+0.21
9	+0.24	+0.11
10	+0.22	+0.13
11	+0.22	+0.11
12	+0.24	+0.18
13	+0.31	+0.21
14	+0.12	+0.22
15	+0.21	+0.19
16	+0.15	+0.18
17	+0.20	+0.22
18	+0.22	+0.22
19	+0.23	+0.23

[1] Eight trials, four under each reinforcement schedule.

Figure 12. Time-Response Curves for Drug Blood Level and SER Effects: Pentobarbital.

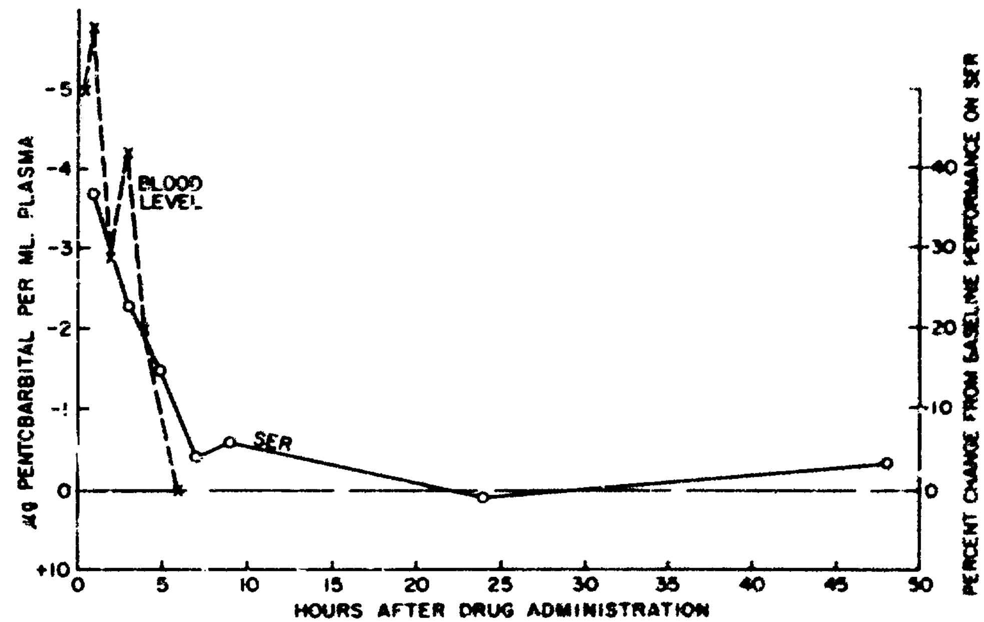


Table 55

Straightaway Approach Response to Differential Reinforcement: Time Scores During Drug Trials

Daily Trial Block[1]	Mean Log Time							
	50% Reinforcement				100% Reinforcement			
	Control	4	8	16	Control	4	8	16
20	+0.23	+0.17	+0.12*	-0.02*	+0.25	+0.19	+0.14*	0.00*
21	+0.24	+0.21	+0.18	+0.06*	+0.22	+0.22	+0.16	+0.08*
22	+0.24	+0.22	+0.20	+0.12	+0.23	+0.23	+0.19	+0.13

[1] Eight trials, four under each reinforcement schedule.

* Significant a p = .05 or better when compared with the control group.

Displacement activities of these kinds have often been reported in studies of behavior under "stress": for example, increases in defecation and urination in a mildly stressful situation have served as measures of "emotionality" in selective breeding experiments (e.g., Hall, 1951); attacks upon the apparatus and upon the experimenter have appeared under conditions of conflict between avoidance response (e.g., Khairy et al, 1957). Stresses of these kinds may also produce oscillation of responding (Miller, 1944) similar to that reported in the preceeding paragraph. Analogies between such characteristic reactions to external changes as evidenced in self-regulating physical, biological and behavioral systems have been pointed out in terms of homeostatic or negative-feedback models (Rao and Russell, 1960). The reactions occur very generally in all species of animals studied, including man.

3. Drug effects. Effects of Librium were clearly apparent one hour after administration. Table 55 shows that the rank order of decrements in performance on day 20, under both conditions of reinforcement, correspond exactly to the rank order of drug doses, i.e., the greater the dose, the greater the decrement. Differences between each of the groups receiving doses of 8 and 16 mg/kg and the control group were significant at $p < .05$ and $p < .001$, respectively, as determined by t-tests. These differences gradually disappeared until on day 24, 48 hours after drug administration, none was statistically significant. These results corroborate those reported earlier in section IV E of the present report, when the straightaway approach response involved only one schedule of reinforcement.

Summary. The results of this pilot study indicate that signs of reactions to stress may be produced in a standardized situation involving a single form of operant response, a straightaway approach response, generated under conditions of differential reinforcement. The signs consisted of marked increases in defecation, urination, squealing, rapid circling movements, and attacks on the apparatus and the experimenter. They were accompanied by oscillation in the SAR-D operant response which took the form of faster responding first to 100 per cent reinforcement, and then to 50 per cent reinforcement; the magnitude of this oscillation decreased as the number of trials increased. The time limitations of the study did not make possible investigation of effects of drugs upon these reactions. Further research would be necessary to determine whether the reactions could be enhanced or prolonged by chemical agents, thus producing more significant incapacitation of behavior.

The effects of Librium on the SAR-D after a relatively stable baseline had been attained and oscillation minimized corroborated dose-response and time-response relations obtained in studies of the SAR under conditions of a single reinforcement schedule.

V. Conditioned avoidance response (CAR) using monkeys as subjects.

One of the objectives of the present project was to extend the study of behavior measures and of research designs to more than one species of animal subjects. Monkeys were chosen as a second species because of their phyletic position closer to man than the rat. This section of the report describes the first experiment using monkeys; because of time limitations it was not possible to pursue as full-fledged experiments other pilot studies begun with this species of subjects.

During the past several years the conditioned avoidance response, CAR, has become one of the most widely used behavior patterns in research on drug-behavior interactions. A number of specific techniques have been developed for the "standard" measurement of the CAR, including "pole-climbing", "shuttle box", "wheel-turning" and various other operant conditioning procedures. In fact the general features of these techniques were described by Warner (1932) as early as 1932 in some of the first studies reporting conditioning in rats. Conditioned avoidance responses appear very frequently in the normal response repetoires of all animals, including man. The possibility of temporarily disrupting such responses deserves investigation in any systematic study of incapacitating chemical agents. The present study was undertaken as a first step toward standardizing a procedure for generating and measuring CARs which could be included in a test battery for screening chemical agents for their potential incapacitating properties.

Research design. The research design involved three experimental treatments, drug doses, which constituted the independent variable. Each animal was studied under all three conditions, the order of treatments being assigned randomly to individual subjects. Librium was the drug used; the three dose levels were: 30 mg/kg body weight administered orally; 2 mg/kg and 10 mg/kg given intraperitoneally. Three measures of behavior constituted the dependent variables: response rate, response-shock rate, and shock-shock rate. In order to obtain information about time-response relations, test sessions were scheduled at regular intervals following administration of the drug.

Test situation. The requirement of the test situation was that the CAR be placed under experimental control in order that it could be investigated as a function of drug administration. The response selected was a key-press which would delay the onset of an electric shock for 30 seconds. The nature of the reinforcement received was response-contingent: by pressing a key within 30 seconds, the shock could be avoided; failure to respond adequately in this time dimension resulted in punishment, which continued at five-second intervals until a response was made.

The test environment consisted of a cage 18 by 14 by 28 inches in its three dimensions. This allowed a subject sufficient space in which to move about, but still made it possible to control experimentally irrelevant variables. The cage was placed in a chamber where such variables as illumination and ambient noise were controlled; both were in an experimental room with thermostatic control of temperature.

A bakelite panel at one end of the cage contained two transilluminated keys 1.5 inches in diameter and an opening into a recessed food cup. Level extensions protruded 0.5 inches into the cage from the bottom of each key and extended 3.0 inches down the panel. The floor of the cage consisted of metal rods through which could be passed a controlled electric current of two milliamperes from a shock generator connected through a standard shock scrambler. The experimental procedure was programmed automatically to provide a 30 second interval between a response and the onset of the next shock; a response on either key delayed the next shock for this interval. When not successfully avoided, the shock was administered at five second intervals.

Responses were recorded on a cumulative work recorder, which kept graphic records of the number of successful avoidance responses, the number of shocks received and the number of responses to the right-hand key. These data provided the bases for determining three parameters of the response: response rate, successful avoidances per minute; response-shock rate, unsuccessful avoidances per minute; and, shock-shock rate, failures per minute to respond during the inter-shock interval resulting in two successive shocks.

Subjects. Six squirrel monkeys, Saimiri sciurea, served as subjects. Three were males and three, females. All were purchased from suppliers who obtained them directly from their native habitats. Their ages were unknown, but all appeared to be relatively young animals. None had participated in any previous research studies. They were housed in separate cages during the experimental period and were fed daily on standard laboratory chow supplemented every other day with 20 grams of a vitamin-protein supplement. Water was available *ad libitum* in their home cages.

Procedure. During the first phase of the study the animals were introduced to the test situation and trained, by the method of successive approximations, to make the avoidance response. Shaping of the response continued for six weeks on a schedule in which test sessions four hours in duration were given at 48-hour intervals.

The first Librium series began 48 hours after completion of the preliminary training. The drug was administered at the start of the first test session of the series, which consisted of eight four-hour sessions at 48-hour intervals. The three drug series were conducted successively in accordance with this schedule, the period between administrations of the drug thus being 16 days. The order of doses administered was randomized for each individual subject.

Results. The results are summarized in Tables 56, 57, and 58. Each table gives the mean rates for the last two test sessions before administration of the drug and for four sessions after administration, by which time performance had returned to predrug baseline levels. The drug was administered immediately prior to the test session numbered "3" in the tables. Because of the pilot nature of the experiment, extensive statistical analyses of the results were not warranted. The statements which follow indicate some of the features of the present CAR procedure to be considered in standardizing it for inclusion in a behavioral screening battery.

Table 56

Conditioned Avoidance Response: Response Rate[1]

Test Session	30 (oral) $\bar{X}$	30 (oral) Range	10 (IP) $\bar{X}$	10 (IP) Range	2 (IP) $\bar{X}$	2 (IP) Range
	Dose (mg/kg)[2]					
1	6.58	3.23-9.93	6.49	3.73-8.05	6.54	4.04-10.21
2	7.86	2.82-11.90	6.44	3.77-9.15	6.67	4.11-10.48
3	3.28	2.17-4.36	3.80	2.47-5.14	6.43	4.43-8.43
4	10.40	3.70-17.10	10.65	3.29-17.99	6.64	4.16-9.05
5	7.16	3.73-10.60	6.86	3.00-10.15	7.02	4.00-9.74
6	6.09	3.98-8.20	6.72	4.40-7.72	6.80	4.00-15.51

[1] In mean number of avoidance responses per minute

[2] Administered at start of session 3.

Table 57

Conditioned Avoidance Response: Response-Shock Rate[1]

Test Session	Dose (mg/kg)[2] 30 (oral) $\bar{X}$	Range	10 (IP) $\bar{X}$	Range	2 (IP) $\bar{X}$	Range
1	0.06	0.02-0.09	0.09	0.04-0.09	0.07	0.00-0.13
2	0.08	0.02-0.12	0.09	0.05-0.12	0.07	0.02-0.18
3	1.06	0.76-1.37	0.82	0.39-1.35	0.10	0.07-0.13
4	0.07	0.02-0.09	0.06	0.03-0.12	0.05	0.00-0.10
5	0.07	0.02-0.10	0.07	0.00-0.10	0.07	0.03-0.15
6	0.08	0.05-0.10	0.08	0.08-0.09	0.08	0.00-0.20

[1] In mean number of response-shock combinations per minute.

[2] Administered at start of session 3.

Table 58

Conditioned Avoidance Response: Shock-Shock Rate[1]

Test Session	Dose (mg/kg)[2] 30 (oral) $\bar{X}$	Range	10 (IP) $\bar{X}$	Range	2 (IP) $\bar{X}$	Range
1	0.01	0.00-0.01	0.02	0.00-0.02	0.01	0.00-0.01
2	0.03	0.03[3]	0.02	0.00-0.04	0.01	0.00-0.01
3	1.23	1.17-1.29	0.55	0.23-0.85	0.00	0.00-0.02
4	0.02	0.01-0.02	0.01	0.00-0.12	0.00	0.00[3]
5	0.01	0.00-0.02	0.00	0.00-0.06	0.00	0.00[3]
6	0.02	0.02[3]	0.02	0.00-0.03	0.00	0.00[3]

[1] In mean number of shock-shock combinations per minute.

[2] Administered at start of session 3.

[3] All measures of the same magnitude.

1. Predrug baseline performance. The predrug training procedure led to very stable baselines of performance for each individual subject. There were inter-individual differences in the levels of performance, as indicated by the ranges for test sessions 1 and 2 in the three tables. As with other behavior measures discussed earlier in the present report, establishment of a stable predrug baseline for each subject is important if each animal is to be used as its own control.

2. Drug effects. Immediate effects of the drug appeared in all three measures of performance within the four-hour test period which followed drug administration. The rate of the CAR during this first post-drug test session decreased markedly; response-shock and shock-shock rates increased. This indicated a shift from the well-established avoidance behavior to escape behavior, which had been almost eliminated in the predrug training period. The typical sequence of behavioral changes following administration of the two larger drug doses was: development of severe motor incoordination, reaching its peak in about 30 minutes; gradual return of coordination accompanied by predominance of escape behavior in which the key-press response was elicited by the shock rather than preceeding it; and final re-establishment of the avoidance behavior as the end of the four-hour test session approached. During the period of motor incoordination it was noted that the animals reacted to the shock by a kind of "freezing" behavior, indicating that the shock was still producing an effect even though not eliciting the key-press response.

Although, as was pointed out earlier, the pilot nature of the study does not warrant extensive statistical treatment, some idea of the significance of the drug effects may be gained from an examination of individual records of performance during a predrug and a drug session. For example, during the last four-hour predrug test session, Monkey No. 1 made a total of 800 avoidance responses. Dividing the session into 24 10-minute segments makes it possible to examine the variability of performance during the four hours: the mean for the 24 segments was 33.33 with a standard deviation of 4.80. This mean rate of 3.33 avoidance responses per minute falls toward the low end of the range of response rates for this last predrug test session, as shown in Table 56. During the four-hour session immediately following administration of 10 mg/kg of Librium, Monkey No. 1 made a total of 521 responses;

the mean for the 24 10-minute segments was 21.71 with a standard deviation of 5.75. Examination of the 24 time segments shows that the significance of the reduction in response rate during the drug period needs no statistical test: in <u>all</u> 24 instances the number of responses under drug was less than the number under predrug conditions. An F-test using unbiased estimates of population variance derived from the variances of the 24 time segments indicates that the intraindividual variability was not significantly altered by the drug. Similar analyses of the number of shocks, i.e., combined response-shock and shock-shock conditions, received by this subject during the same two test sessions support the general description of drug effects given above: during the last predrug session Monkey No. 1 received a total of 39 shocks at a rate of 0.16 per minute; under drug the total increased to 514 and the rate to 2.14 per minute. In <u>all</u> 24 time segments the number of shocks received under drug was greater than the number received under predrug conditions. Here there was a significant change, i.e., increase, in intraindividual variability, the F-ratio using unbiased estimates of population variance giving a $p < .005$. Had a standardized CAR procedure suitable for regular use with squirrel monkeys been already established, the data from all subjects would have been obtained under identical conditions and, therefore, could have been combined for a final statistical analysis. The present pilot study has provided a procedure which can be used in a standardized way for future studies.

One particularly apparent effect was the enhancement of the CAR response rate during the test period 48 hours after drug administration. The basis for this "rebound effect" did not appear to lie directly in the drug effects. In the present test situation it was possible to produce the effect by delaying the first postdrug test session for 72 to 96 hours or by physically restraining the animal from making the key-press response in the non-drugged state, i.e., by shortening its leash to the extent that it could not reach the keys. It may be that the rebound effect follows a period during which the CAR is restricted from occurring; in the present study administration of the drug may be viewed as having resulted in such "restriction" in the sense that it was followed by a period of motor incoordination during which the key-press response did not occur.

3. Dose-response relations. Relations between dose levels of the drug and changes in performance are shown in Tables 56, 57, and 58. The drug dose specified was administered at the beginning of test session 3. For all three measures the magnitudes of changes after administration follow the same rank order: 2 mg/kg IP, 10 mg/kg IP, and 30 mg/kg oral. Little, if any, drug effect was apparent in performance during the 2 mg/kg IP series. During the other two series all subjects showed depressions of response rate and increases of response-shock and shock-shock rates. The fact that the order of the drug effects was the same for all measures of performance and that all subjects showed the effects is evidence that the dose-response relations were systematic and significant.

4. Time-response relations. Drug effects on the three measures of behavior disappeared within 48 hours after administration, i.e., by then performances had returned to predrug baseline levels. This duration of effects was in the same order as the durations of effect for Librium reported earlier for other measures of behavior studied in the present project.

The rebound effect observed in measures of CAR response rate appeared only during the test session which began 48 hours after drug administration. It had disappeared by the beginning of the next test session, i.e., 72 hours after administration. It should be emphasized again, however, that the effect itself may be only indirectly related to the presence of the drug.

5. Variability of performance. The interindividual variability of performance during each of the test sessions reported here is described in terms of ranges of performance measures and is given in Tables 56, 57, and 58. Following administration of the drug, the range decreased for the response-rate measure and increased for the other two measures.

Summary. The pilot study of the conditioned avoidance response, CAR, was conducted as a means of standardizing a procedure for inclusion in a behavioral test battery using the squirrel monkey as a subject for screening potential incapacitating agents. The results showed that stable baselines of performance could be established and that these baselines were differentially sensitive to different dose levels of the experimental drug, Librium. The effects, when present, had time-response characteristics similar in nature to those of effects produced by Librium in other measures of behavior.

K. Other pilot studies

Other pilot studies were begun, but did not progress sufficiently in the time available to provide other than very preliminary data. These studies will be described only briefly in the following paragraphs.

1. General activity. The behavior of living organisms includes the presence of a considerable amount of movement and general activity which is not immediately understandable in terms of specific stimulus or motivational conditions. Numerous studies have been made of the extent of this general activity, of conditions within and external to an organism which may affect it, and of its possible relations to genetic factors and to specific physiological drives, e.g., hunger, thirst, sex. The studies have shown that general activity is markedly affected by administration of certain chemical agents and that it may be affected when other behavior patterns are not significantly altered. In so far as general activity reflects an organism's level of activation (Duffy, 1962) it may serve as a useful measure in screening for the effects of incapacitating agents.

The measurement of general activity presents some technical problems which, after study, led us to develop a new apparatus for more consistent and stable recording. Our first models were developed for measuring general activity of the white rat. The cage housing the animal was placed in a sound-deadened box. It was suspended by a heavy bar over a fulcrum. The transducing element, a piezo-electric device called a "Bimorph" (Brush Electronics, Inc.), was fixed to the bar at the stress point over the fulcrum. Movements of the animal within the cage caused the bar to flex slightly, which, in turn, flexed the Bimorph whose output was a small voltage spike proportional to the degree of flexion. The flexion required was so small as to produce no detectable movement in the cage. The spikes of voltage generated were amplified and a three-channel print-out counter registered the number of spikes which met three separate voltage level criteria, representing "high", "medium" and "low" levels of activity. Because of the sensitivity of the apparatus, the three levels could be adjusted so that ranges of activity from sniffing to violent jumping could be monitored and recorded in their appropriate categories. By making food and water constantly available in the cage, recordings could be made over extended periods of time without disturbing the animal. Measures of activity could be printed out automatically at any predetermined interval, e.g., minute-to-minute changes, cumulative hourly measures, etc.

Trial runs were carried out under normal control conditions and following administration by intubation of Librium at doses of 16 and 32 mg/kg body weight. The results showed a trend for activity to decrease initially following administration, the decrease being greater the greater the dosage, and for recovery to occur as expected. The trials also revealed some inadequacies of the apparatus, centering around the transducing elements and the required method of mounting them; there were no problems in handling the voltage spikes once they were produced nor in automatically recording them in appropriate categories. The inadequacies were due to mechanically produced resonances in the system, uncontrollable changes in leverage forces on the transducing element, and the lack of means for relating activity records to actual physical forces involved in their production. The next step in developing the apparatus should involve the testing of more directly activated transducing systems. One possibility under consideration involves transducers of the pressure activated type, which permit the direct mounting of the activity cage on the transducer itself. This would permit more direct measurement of forces involved in the animal's movements. The transducers would also lend themselves more readily to calibration that would relate the recorded data to the actual physical forces involved in various kinds of activity.

When adequately developed, this concept of recording general activity should provide a standard means of measuring this form of behavior in a wide range of animal species, including man, and of analyzing the behavior into a variety of components describable in terms of changes in actual physical forces involved.

2. Spontaneous alternation. Since forms of apparently unlearned behavior may be affected by administration of chemical agents, some preliminary trials were run of the effects of Librium on "spontaneous alternation". This form of behavior received considerable attention a number of years ago (e.g., Dennis, 1939) and has enjoyed a recent revival of interest in tests of statistical models of behavior.

The test situation employed was a Y-maze. Each trial began with the placement of the animal in the base section of the Y and ended when he reached the end of one of the two alleys beyond the choice point. Two trials were run each day, with a maximum allowable time of one minute for each trial. Food was available in each alley. All animals were pretrained to eat in the maze; they were given food and water _ad libitum_ for one hour after their daily trials and then deprived of both for 23 hours before the next day's runs.

Alternation behavior was measured every 24 hours in two-trial sequences. The animal was allowed to eat for 15 seconds at the end of each trial. Following trial 1 he was returned to his home cage and one minute later placed in the maze for his second trial of the day. Under these conditions alternation behavior stabilized at 85-90 per cent alternation during the first week and did not change significantly during the remaining two weeks of predrug testing. Intraperitoneal injections of placebo produced no effects upon alternation behavior when injected animals were compared with normal control animals. Termination of the project prevented implementation of a Latin Square design for studying dose and time response relations and carry-over effects.

3. Conditioned emotional response (CER) using monkeys as subjects. In implementing the plans of the project to study more than one species of infrahuman animals, work was begun on the design of equipment and procedures for establishing CERs (Hunt, 1959) using squirrel monkeys, _Saimiri sciurea_, as subjects. The test situation consisted of a ventilated "Skinner" box, 1 ft. x 1 ft. x 1 ft. in interior dimensions, constructed of aluminum double walls separated by 1 in. of fiberglass. On one panel were mounted two house lights, a signal light, a minipulandum bar, and a reinforcement delivery tube. A reinforcement reservoir and delivery system, a speaker, and a relay to provide auditory feedback for bar presses were attached to the back of this panel. The floor of the box consisted of quarter inch aluminum rods spaced on one inch centers through which electric shock could be delivered to the subject. The behavioral contingencies were programmed by automatic electromechanical devices. All apparatus producing auditory stimuli were isolated in another room.

Six monkeys served as subjects during preliminary work to establish satisfactory procedures for generating the CER in this species of subjects. Attention was directed toward study of various key parameters affecting establishment of stable bar-pressing and suppression responses, including nature of reward reinforcers, VI schedules, and duration, frequency and scheduling of the CS and shock reinforcement for maximizing the suppression effect. Investigating such parameters, important in standardizing a final procedure, is time-consuming and was not completed during the period of the project.

V. RELATIONS BETWEEN PHARMACOLOGICAL EVENTS AND BEHAVIOR

The pharmacological studies undertaken in the course of this present research were concerned with two matters of basic importance in studies of drug-behavior interactions. The first involved the establishment of standard conditions for the administration of each drug in the experiments on behavior; the conditions which were standardized included: dose range, route, drug vehicle or preparation, and time intervals after drug administration for observation of maximum behavioral effects. The second concerned the determination of time curves for changes in the blood levels of intact drug after administration under the standardized conditions. Information about the time characteristics of the absorption and metabolism of a drug is essential in determining whether any concommitant changes in behavior are related to the presence of the drug in the body or to alterations in biochemical systems induced by the drug. The first of these considerations was essential to the design of the experiments on behavior in order to obtain reproducible, interpretable data, uncomplicated by drug effects on gross sensory or motor responses. The blood-level curves constituted the first basic data of a biochemical nature needed for interpretation of the mode of action of each drug.

The three drugs studied, i.e., Librium, pentobarbital and ethanol, constituted an interesting comparison of "tranquilizing-type" drugs with varying degrees of psycholeptic, sedative, and hypnotic properties. The results of mass clinical trials suggest that these drugs have differential effects in the treatment of different kinds of behavior disorders.

As in the case of several of the experiments described earlier, the studies to be reported in the following paragraphs are in the nature of pilot investigations. In the eighteen months available for research the project could be oriented toward only one major aspect of drug-behavior interactions. The experiments on behavior were chosen as the central focus and all other work had to serve in a supportive role.

A. Selection of dose levels

Decisions about drug dose levels, based upon some empirical data involving the specific strain of animals to be used in the main experiments on behavior, had to be made before the experiments could begin. These data had to be approximations from a very minimum number of observations if the main body of studies were not to be delayed beyond a possibility of completion. The results obtained, though approximate, were adequate to establish for each drug a usable "order of magnitude" for the maximum dose to be administered. With this dose level established, decisions could be made about the distribution of experimental dosages within the range from it to a "zero dose" or placebo. The results also provided information about the feasibility of the techniques employed for routine use with larger groups of animals in later, more precise studies of the kind described as "Phase 1. Preliminary screening: animal" in the project's Report No. 1.

No attempt was made to determine median lethal doses, LD_{50}'s, for the three experimental drugs. Such values could be determined at a later date, using the standardized conditions of drug administration and employing well-established methods for their determination. For comparative purposes in Figure 8 and Table 60, the LD_{50} value of 2000 ± 400 mg/kg for oral administration of Librium in the rat was used, a value supplied by Dr. J.J. Pepper from data obtained in the Hoffman-LaRoche Laboratories.

BD_{50} determinations for gross behavioral responses. In order to set the experimental dose levels to cover the range over which changes in behavior might occur, determinations of BD_{50}'s were made using four gross behavioral responses. These included two measures of motor coordination, i.e., the righting reflex and gait or pattern of locomotion, and three measures of response to "stress", i.e., startle response to air puff on dorsal hair, st, startle response to loud noise, N, and response to the pain of a tail pinch, P. All determinations of the present series were made by the same investigator. For each of these measures a "normal" response was defined by the investigator on the basis of observations on untreated animals. During a test session responses were rated on a five-point scale:

0	1	2	3	4
no response		normal response		greatly exaggerated response

Observations were made during test sessions at selected time intervals after administration of a drug; averages of individual ratings were computed for each dose level studied at each time interval. A "biologically significant change" in the gross behavior measures was arbitrarily defined as deviation from the "normal range" of 2 $\pm$ 1 on the rating scale.

Table 59 illustrates the results obtained from the application of this procedure. The table summarizes data from two experiments with Librium as the drug. The data presented are mean ratings for groups of rats. They indicate that a dose of 32 mg/kg avoids detectable gross behavioral changes and should be a safe maximum level for use in the detailed behavioral studies. The results also indicate that the stress responses to noise and pain were more sensitive to Librium action than the motor responses as measured by righting reflex and walking ability.

From raw data such as these, the conditions were set for preliminary studies of each drug in the detailed experiments on behavior. The conditions are summarized in Table 1 presented in an earlier section, page 18. In actual practice, it was occasionally necessary to modify these preset conditions. For example, it was necessary to increase the maximum dose of pentobarbital to a level much higher than that producing effects on certain of the gross behavioral measures in order to achieve significant changes in the detailed behavioral studies. Another example illustrates how theoretically optimum conditions may be limited by characteristics of the subjects to be studied. More marked effects of ethanol on the detailed behavior measures might have been

Table 59

Ratings of Gross Behavioral Responses[1] after Acute Oral Administration of Librium to the Rat

Dose (mg/kg)	Time Interval after Drug Administration: 30 min.				1 hour				2 hours				6 hours			
	R	W	N	P	R	W	N	P	R	W	N	P	R	W	N	P
100					1.7	1.7	0	0.3					2	2		0.6
75					1.7	1.7	0.7	0.7	2	2		1.5	2	2		1
50	2	1.7	0.7	0.3[2]	2	2	0.6	1.3	2	2		1.7	2	2		1.7
32	2	2	1.7	2	2	1.5	2		2	2	2		2	2	2	
16	2	2	1.7	1.7	2	1.5	2		2	2	2					
8	2	2	1.5	2	2	1.5	2		2	2	2					
0	2	2	2	2	2	2	1.7	1.7	2	2	2					

[1] Test measures are : R - Righting reflex
W - Gait or walk
N - Noise response
P - Pain response

[2] Underlined ratings represent biologically significant changes from a pre-defined normal range of 2 ± 1.

observed if there had not been a physical limitation to the amount of drug which could be safely administered to the rat via oral intubation. The oral route was selected a priori as the route of choice for all the detailed behavioral studies reported here. For purposes of comparison, conditions for intraperitoneal drug administration were determined and are reported in Table 1.

No attempt has yet been made to standardize or characterize these "quick and dirty" preliminary gross behavioral observations with respect to reliability, validity, or intra- and interindividual consistency. This will inevitably be done when a more comprehensive final preliminary screening procedure is set up on a larger scale with adequate facilities.

Determination of drug profiles. Dose-response data for mortality, gross behavioral effects, and effects on specific behavioral measures can be expressed on the type of log-probability plot discussed earlier, to give a type of drug-behavior profile for each drug studied. Figure 8 shows this type of plot for Librium in which the per cent of significant effect is expressed as a function of dose level. The "lines of best fit" are estimated visually among the 3 or 4 points plotted for each effect tested. Even taking full account of the approximate nature of the toxicity and gross behavioral data available so far, it is readily apparent that, for Librium, the dose ranges producing toxic effects and behavioral effects are of different orders of magnitude. Further, there is a clear separation between effective dose ranges producing significant gross behavioral effects and effects on the detailed behavior measures. From such plots as this LD_{50}'s and BD_{50}'s for each behavior measure can be calculated.[50] LD_{50}'s and BD_{50}'s, corresponding to drug levels which are 5 per cent effective in producing mortality and behavioral effects, can also be determined. Table 60 summarizes such approximate values obtained for the drugs studied in the present project.

Table 60

Comparison of Selected Effective Dose Levels for Librium, Pentobarbital, and Ethanol

Dose	Route	Lethal Dose LD_{50} (mg/kg)	Motor Response ED_{50}	Motor Response ED_5 (mg/kg)	Stress Response ED_{50}	Stress Response ED_5 (mg/kg)	Behavioral Battery ED_{50} (mg/kg)	Test Response ED_5
Librium	Oral	2000*	100	60	50	35	8.5	1.1
	IP	450*	60	22	16	6	---	---
Pentobarbital	Oral	(>40)	20	12	5	3	12	2.2
	IP	(>40)	14	11	---3	---3	---	---
		(ml/kg)	(ml/kg)		(ml/kg)		(ml/kg)	
Ethanol	Oral	(>3.0)	(>3.0)		3.0	2.2	1.5	0.4
	IP	$(<1.0)^2$	1.0	0.6	0.4	0.25	---	---

1 LD_{50} values for Librium taken from private communication from Dr. J.J. Pepper, Hoffman-LaRoche Laboratories.

2 Delayed mortality (24-72 hours) allowing gross behavior measures at lethal levels.

3 Response ratings confused by simultaneous observation of calming and excitatory effects in replicate animals.

It is of interest to note the appreciable differences for Librium between the effective dose ranges for the four effects studied, particularly the difference between the overall average ED_{50} for the test battery of approximately 8.5 mg/kg and the motor and stress response ED_{50}'s of 60 and 35 mg/kg, respectively. This appears to clearly separate the sedative and/or hypnotic effects from the effects observed on the behavioral test battery. In contrast, pentobarbital shows an overlap among all the effective dose ranges for motor response, stress response, and behavioral test battery response, with stress responses, i.e., startle and pain, being equally or more sensitive than the responses measured on the behavioral test battery. For this drug, then, it is apparent that effects on performance in behavior tests cannot be separated from the gross hypnotic and sedative effects.

B. Drug absorption and metabolism studies

The reasons for obtaining information on the time characteristics for absorption and metabolism of drugs used in experiments on drug-behavior relations have already been given. In the present project it was possible to make determinations of drug concentration changes in blood only; tissues, particularly brain, are scheduled for study later. One of the several reasons for limiting determinations in this way was the fact that circumstances permitted determinations for Librium using both rat and human subjects: the rats could be studied under the standard conditions of the present experiments on behavior; blood samples could be drawn from human subjects participating in experiments in another university laboratory. Data from these two sources could be used for purposes of comparison.

Librium. Librium, 7-chloro-2-methylamino-5-phenyl-3H-1, 4 benzodiazepine-4-oxide hydrochloride, was made available through the Hoffman-LaRoche Laboratories. All work with rats was done with pure crystalline Librium hydrochloride. The human studies were carried out with 25 mg. capsuls designed for oral clinical administration. These contained inert carrier solids to a total weight of 200 mg. per capsul, which did not interfere with the colorimetric determination of Librium in dilute solution.

Methods of analysis. Two methods for the quantitative assay of Librium in blood extracts have been used in these studies. In the earlier work with human blood samples, a colorimetric procedure was used which had been designed for routine clinical assay of Librium in blood or urine and supplied to us by Dr. L.O. Rnadall of Hoffman-LaRoche Laboratories. In the later work with rat blood, we had available a double-beam recording spectrophotometer, Brasch & Lomb, Spectronic 505. This allowed us to develop a more specific assay for intact Librium in dilute solution or blood extracts by utilizing the UV spectral differences between Librium and its hydrolysate, 2-amino-5-chlorobenzophenone, in acid solution. Since this procedure was developed during the course of this study, it is presented here in some detail.

Colorimetric determination of Librium. The procedure made available by Dr. L.O. Randall of Hoffman-LaRoche involved drug extraction from alkaline solution into ether and back-extraction into 6N HCl; acid hydrolysis of the extracted drug, to yield 2-amino-5-chlorobenzophenone; and colorimetric determination of the liberated amine by diazotization and coupling with N-(1-napthyl)-ethylene diamine dihydrochloride, Bratton-Marshall reagent. The method had a reported limiting sensitivity of 2 µg Librium.

We found it possible to increase the sensitivity of the method four-fold by making the following procedural changes: reduction of the acid concentration in the extraction and hydrolysis steps; hydrolysis under milder conditions of temperature as well as acid concentration; reduction of the total volume during diazotization and coupling; and extension of the time allowed for color development.

Spectrophotometric determination of Librium by the optical density difference, O.D.D., method. As shown in Figure 9, the characteristic absorption spectra of Librium in dilute acid shows two diffuse maxima at approximately 250 and 310 mµ. The acid hydrolysate of Librium shows a smaller single maximum at 260 mµ. By comparing an acid extract of Librium against its own hydrolysate as reference solution (curve 3, Figure 10) it is possible to detect well-defined peaks at 244 and 313 mµ which are apparently characteristic of the part of the Librium molecule which is labile to the conditions of hydrolysis. The heights of both peaks bear a quantitative relationship to the concentration of Librium in solution.

Five ml. heparinized plasma is made alkaline with 0.05 to 1.0 g. K_2CO_3. This is extracted with 15 ml. ether by shaking at room temperature for 30 minutes. After centrifugation, 10 ml. of the ether is back-extracted with 5 ml. 3N HCl by shaking for 10 minutes. After aspiration of the excess ether, the acid extract is made up to 6 ml. volume with 3N HCl.

A 3 ml. aliquot of the extract is heated in a boiling water bath for 45 min. After rapid cooling in a cold water bath, it can be made up to its initial volume with 3N HCl.

On a double-beam recording spectrophotometer, the ODD curve (from 220 mμ to 350 mμ) is determined for the 3 ml. aliquot of Librium extract, using the 3 ml. extract hydrolysate as reference solution. The Librium concentration is proportional to the height of the maximum at 244 mμ after subtracting the baseline minimum at 284 mμ. Above a Librium concentration of 6 μg per ml., this proportionality is .061 ODD units per μg per ml. Below a Librium concentration of 6 μg per ml., this factor decreases to the limiting sensitivity of the method of .025 ODD units at 1 μg per ml. Since the extraction procedure gives an overall Librium recovery of 75 percent (for Librium added to untreated rat plasma), this method has a limiting sensitivity of approximately 1.5 μg Librium per ml. plasma.

In Figure 10 are shown characteristic curves of plasma extracts taken at 0, 3, and 12 hours after acute oral administration of Librium at 32 mg per kg in the rat. As can be seen, the curve for the highest blood level at 3 hours is not qualitatively different from the curve for the lowest level at 12 hours. In our results to date, there is no consistent evidence for metabolically-induced changes in chemical structure of circulating Librium which can be detected by this procedure.

Acute rat studies. At time intervals of 1, 3, 5, 7 and 12 hours after acute oral administration of Librium at 32 mg/kg replicate rats were sacreficed via ether anesthesia, and blood samples were withdrawn via the vena cava. Librium levels per ml. plasma were then determined by the spectrophotometric "optical density difference" method. The time intervals were selected to correspond with the smallest intervals used in the behavioral tests.

Results of this work are summarized in Table 61 and Figure 11, where it can be seen that the peak drug level in the blood is reached at 3 hours.

Table 61

Librium Blood Levels after Acute Oral Administration at 32 mg/kg in the Rat

Time after Treatment[2] (Hours)	Blood level Mean ± S.D.[1] (µg/ml. plasma)	No. observations
1	4.0 ± 1.03	5
3	6.7 ± 3.45	3
5	4.4 ± 1.48	3
7	2.2 ± 0.62	2
12	1.0 ± 0.59	3
24	tr.	2

[1] S.D. estimated from range according to the method of Dean and Dixon (1951).

[2] Librium administered in aqueous solution at 32 mg/ml.

Chronid human studies. Using the colorimetric (hydrolysis-diazotization) procedure for the determination of Librium level in the blood, a study was made of human subjects on a chronic administration schedule. On a dosage of 100 mg per day (25 mg. q.i.d. per os), two subjects attained a blood level of approximately 4 µg per ml. plasma within 3 days. On cessation of treatment, blood levels of the drug dropped off to zero over a period of 5 to 7 days. Data for these two subjects are shown in Table 62. A single blood analysis on one subject who had been on continuing Librium therapy for 60 days at a daily dose of 30 mg (10 mg t.i.d.) showed a blood level of 1.9 µg per ml. plasma.

Concurrent physiological measures taken on the subjects, AR and MS, supported the general conclusions drawn from a much larger study (Technical Report No.7 Contract Nonr. 908-15 with Indiana University, R.W. Russell, Project Director, August 1961. "Effects of Chronic Administration of Librium and one of its Analogs on Human Somatic Responses.) Librium appeared to effect a decrease in heart rate and EMG and an increase in skin resistance. Gastrointestinal motility and respiration rate were unaffected. In general, the startle response to noise of each of these indices was suppressed by Librium treatment. With the subjects AR and MS, some of these observed effects showed a delay in recovery to predrug levels of 1 to 2 weeks, although, as previously noted, Librium blood levels were below the limits of detection within one week. This lack of parallelism between time-response curves for blood level and effect is similar to that observed in the acute rat studies as shown in Figure 11.

Pentobarbital. Pentobarbital sodium was prepared in aqueous solution within three hours of the time of administration. Pentobarbital blood levels were determined according to the Method of Bush (1961) which includes preliminary extractions into butyl chloride and alkaline buffer, followed by quantitative determination in a UV double-beam recording spectrophotometer via the ODD (optical density difference) maximum at 240 mµ. The buffer extract at pH 10.0 is read against a reference solution of the extract adjusted to pH 6.25. In our hands this method had a limiting sensitivity of .03 ODD units for 1 µg pentobarbital per ml. of buffer extract, with a recovery factor of 75 percent for pentobarbital extraction from whole blood.

Table 62

Librium Blood Level Studies: Human Subjects

Subject	Treatment			Librium Level*
	Daily Dose (mg)	Days on Drug	Days off Drug	(µg/ml. plasma)
I: AR	100	1	---	0.3
		5	---	4.5
		5	2	1.75
		5	5	0
VI: MS	100	1	---	0.4
		3	---	4.0
		6	---	3.6
		6	4	0.9
		6	7	0
V: H	30	60	---	1.9

* Librium determined by hydrolysis and diazotization procedure.

Acute rat studies. Conditions for obtaining blood samples in the pentobarbital experiments were the same as those described in the Librium blood level studies. The results obtained are summarized in Table 63, and are presented graphically in Figure 12, where behavioral effects on the SER are shown to vary directly as the blood level of the drug.

Table 63

Pentobarbital Blood Levels after Acute Oral Administration at 40 mg/kg in the Rat

Time after Treatment[2] (Hours)	Blood level Mean ± S.D.[1] (µg/ml. plasma)	No. observations
0.5	5.0 ± 1.77	3
1	5.8 ± 0.30	3
2	3.0 ± 1.18	3
3	4.2 ± 0.41	3
4	2.0 ± 0.44	2
6	(trace)	2

[1] S.D. estimated from range according to the method of Dean and Dixon (1951).

[2] Pentobarbital administered in aqueous solution at 40 mg/ml.

C. Summary

The results obtained here demonstrate the essential advantages of securing pharmacological and biochemical data under conditions which parallel those used for the behavioral tests. The type of dose-response profile shown in Figure 8 for Librium can detect and emphasize differences among specific drugs. For example, the dose-response profile for Librium shows three discrete families of curves, each encompassing a different dose range: i.e., a group of curves for specific behavioral effects at doses below 32 mg/kg, another group of curves for somatic responses or "gross behavioral effects" at doses between 50 and 100 mg/kg and the curve for mortality effects at doses above 1000 mg/kg. This is in contrast to the profiles for pentobarbital or alcohol, where the curves for specific behavioral effects and "gross behavioral" or somatic responses all occur within the same dose ranges. It might be postulated that such separation of behavioral and gross somatic effects as shown by Librium is a distinguishing characteristic of a "tranquilizer"-type drug as contrasted to primarily sedative or relaxant-type drugs.

A differentiation between Librium and pentobarbital is also possible on the basis of comparison of the time-response curves of behavioral effects and drug blood levels as shown in Figures 11 and 12. For pentobarbital, it appears that behavioral effects as measured on the SER are dependent on the presence of the drug. For Librium, these behavioral responses persist well beyond the disappearance of intact drugs from the blood stream. Such results further serve to emphasize the need for additional parallel biochemical studies, which would allow comparison of time-response relationships between behavioral effects and effects on selected CNS biochemical systems, possibly those involving acetylcholine, serotonin, and/or norepinephrine.

VI. SUMMARY AND DISCUSSION

Nature of the project. The present project arose from the wish of the U.S. Army Chemical Center to obtain information about factors which affect the behavioral testing of chemical agents for incapacitating properties. "Incapacitation" is defined in terms of changes in behavior, changes which disrupt the organization and performance of normal activities. The final criteria for testing incapacitating agents must, therefore, involve measures of behavior. Such measures may be affected by the characteristics of the organism under study, the conditions under which behavior is generated for test purposes, the research design employed in the test, and the properties of the chemical agent administered. From the practical point of view these variables contribute to the risks of labelling a new agent as worthy of development when in fact it is not or, on the other hand, of failing to identify a new agent which is promising.

The contract for the project covered a two-year period of work. In accordance with its specifications, the first six months were devoted to examining the objectives of a program for identifying and evaluating potential incapacitating agents. The objectives were stated and requirements for achieving them specified in the project's Report No. 1 submitted in September 1960. The report outlined a general behavioral screening program consisting of four phases, two of which involve infrahuman animals and two, human subjects; the first three phases employ laboratory tests and the fourth, field tests:

Phase 1. Preliminary screening: animal.
Phase 2. Screening in depth: animal.
Phase 3. Laboratory tests: human.
Phase 4. Field tests: human.

The final year and a half of the contract was devoted to empirical studies of factors affecting the development and operation of Phases 1 and 2 of the program, using infrahuman animal subjects. The species employed in the research were rats, Holtzman Albino Stock, and monkeys, Saimiri sciurea. After discussions with the Contract Project Officer it was decided that the needs of the project could best be met and its unclassified status maintained if psychoactive chemical agents already in general use were employed in the various experiments; Librium, pentobarbital sodium and ethanol were selected. In addition to experiments on behavior, the project included studies of factors affecting the selection of dose levels and also some limited studies on drug absorption and metabolism.

Preliminary screening. Preliminary screening was necessary in order to set the experimental dose levels for each of the drugs used in the project. Since behavior may be very sensitive to drug action, significant changes in behavior may occur at any dose level between zero and the level at which toxic effects to the body appear. Median effective doses were determined for each of five gross behavioral variables: two measures of motor coordination and three measures of response to "stress". The data from these determinations also provided information about minimum effective doses below which no gross behavioral effects could be observed. These doses were used as guides in setting the maximum dose levels for the more quantitative experiments on behavior; zero dose, or placebo treatment, defined the other end of the working range. Doses within this range were related in decreasing fractional steps of one-half between succeeding dose levels, a selection which proved empirically to be effective with the drugs studied for describing dose-response characteristics and for determining the median behavioral doses, BD_{50}s, of the measures of behavior studied. The processes of selecting dose levels in this way established the range through which the principle independent variable, drug dose, would be systematically varied in the quantitative studies to follow in phase 2.

Research design. Research design is particularly important in studies of the present kind because of the number of variables capable of influencing any set of observations. One of the objectives of the project was to examine empirically the operation of several different research designs. Three were studied in detail: randomized groups design, Latin square design, and Latin square design with replication of the same square. These designs were selected for very practical reasons. It is obviously uneconomical to undertake the costly training of an animal only to use it for a single drug treatment, as in the case of the randomized groups designs used in the present project. It is also obviously impractical and uneconomical to subject an animal to several drug treatments if significant interaction effects between treatments make later interpretation of results uncertain, as is often true when research is designed in such a manner that statistical tests cannot be made for carry-over effects. The Latin square designs provided for such tests. This very fundamental issue, which is often ignored in so-called "practical" screening procedures, will be discussed again later in this summary when it can be illustrated with data from experiments conducted during the project.

Measures of behavior. The primary role of incapacitating agents is to produce temporary deterioration or disruption in the performance of skills which have already been established. Performances which it may be militarily desirable to alter range from simple motor skills to complex decision-making. In the present project, experimental test situations were selected so as to provide as wide a range of different behavior patterns as could be studied during the limited period of the contract. These behavior patterns constituted the major dependent variables of the experiment; they were: food intake, water intake, straightaway escape response, straightaway approach response, fixed-ratio operant response, variable-interval operant response, conditioned emotional response, timing behavior, conditioned avoidance response. The general procedure followed in all the experiments was: to establish stable baselines of performance during a predrug training period; to administer the agent under study at the beginning of the drug period; and, to measure performance at predetermined intervals thereafter until the predrug baseline had once again been firmly established. In this way the full course of any drug effects on performance could be observed.

Behavioral screening programs for chemical agents of any kind must provide quantitative data, the analysis of which will allow statements to be made with confidence that the level of performance has been significantly changed or its variability altered. Agents may produce subtle, yet significant, changes in behavior which are not readily detected by gross observation; such effects, which should be of particular interest to those concerned with incapacitation, can only be studied when quantitative measures of behavior are available to provide data suitable for appropriate statistical analysis. If an agent is to be used for practical purposes, it is also essential that precise information on various parameters of the drug-behavior interaction, e.g., dose- and time-response relations, also be available. Measurements of behavior patterns generated in the test situations listed above provided data which can be used to illustrate the kinds of basic information which should be specified in any program designed to determine the behavioral effects of incapacitating agents.

Results. Report No. 1 described certain desirable characteristics of incapacitating agents. The sections which follow use these characteristics as headings in discussing results obtained from empirical studies conducted during the present project.

1. Predrug baseline performance. Because individual differences characterize the behavior of even the most homogeneous strains of animals, it is desirable to use each subject as its own control. In experiments conducted during the present project the measurement of predrug performance provided baselines which served as reference points for the analysis of drug effects on both performance level and variability. Training was continued until predrug performance had stabilized at an asymptotic level and variability within a test session was minimal. Each animal's performance during the drug phase of an experiment was then expressed as a per cent of its mean level of stabilized predrug performance.

2. Dose-response characteristics. Incapacitating agents should produce their effects on behavior at relatively low dose levels. Although the drugs used in the present project were not "incapacitating agents", the procedures used to determine their dose-response characteristics are generalizable to the study of any psychoactive chemical agent.

By testing the significances of differences in performance at peak effect times for groups receiving different drug doses, it was possible to answer the question as to whether or not a drug had affected behavior within the range of doses administered. The results of the present project clearly illustrated two kinds of differential effects produced by chemical agents:

a. An agent may affect certain behavior patterns and not others. For example, within the standard doses range for Librium there was no evidence that the magnitudes of food and water intake were affected, while other kinds of performance, e.g., operant escape responses, showed very significant decrements.

b. One parameter of a behavior pattern may be affected independently of another. This kind of differential effect is illustrated by the experiments on the straightaway escape response where level of performance was significantly decreased within the standard doses range for all three drugs studied, while variability of performance was affected only during the pentobarbital series. Since incapacitation may be produced when either or both of these parameters is affected, it is important to analyze both during a screening program.

Dose-response characteristics may be described in terms of concomitant variations between magnitude of behavior changes and differences in dose levels of a drug. The typical relation between these two variables in experiments where behavior was significantly affected appeared as increased decrements in performance as dose level increased. The relation operated after a threshold for change was reached; below the threshold, changes in dose level produced no effects upon behavior.

3. Time-response characteristics. There are two main features to be considered here. First, it is generally desirable that the onset of effects occur in one hour or less following administration of an agent, although an agent which had a delayed time of onset would be of interest for special purposes. Second, it is important to know the time course of effects once they have been produced, if an agent is to be put to practical uses where the deviation of its effective action must be predictable.

For all three drugs studied in the present project, it was typical that peak effects were rapidly obtained, i.e., in less than one hour, after drug administration. Above the threshold dose required to produce a change in behavior, duration of effect varied systematically with dose level: duration increased as level increased.

4. Reversibility of effects. It is desirable that the effects of an incapacitating agent at the dose levels required to produce significant changes in behavior be completely reversible, i.e., that the agent produce no lasting behavioral or organic effects. All experiments of the present project were designed to study such reversibility at the behavioral level.

The concept of "incapacitation" developed in Report No. 1 laid emphasis upon the fact that the behavior patterns of living organisms are correlated, directly or indirectly, with biochemical events occurring within the organism; chemical agents may be used to interfere with these biochemical events, thus producing changes in normal behavior. This concept involves three classes of variables - chemical agents, biochemical events, and behavior - all of which may, under specified experimental conditions, exhibit the characteristic of reversibility: drugs are absorbed and then metabolized or eliminated; biochemical events may deviate from their normal resting or steady states and later reestablish them; behavior may change from its predrug baseline and return to it. A systematic body of knowledge about incapacitation, from which predictions could be made as to where to search for new agents, would seem to depend upon information concerning interrelations between these three classes of variables.

During the eighteen months of experimental work under the present project it was possible to make only a small beginning to the task of studying such interrelations. Studies were limited to the determination of certain drug parameters, e.g., ED_{50}s, time characteristics of absorption and metabolism. The results of these studies are reported in detail in Section V of the present report.

On the other hand, information was obtained about the reversibility of behavioral effects for all behavior patterns studied. In all instances where performance deviated from predrug baseline levels after administration of a chemical agent, baseline levels were reestablished, usually within 48 hours, thus indicating complete reversibility of all behavior effects produced by drug administration.

Comparisons were made between the time-response curves of certain of the reversible behavioral effects and drug blood levels. Using the straightaway escape response as an example, it appeared that the behavioral effects were related to the presence of pentobarbital in the body, but that they persisted well beyond the disappearance of intact Librium from the blood stream. The existence of significant carry-over effects for this behavior pattern during a series of weekly administrations of Librium was in contrast to the lack of such effects during repeated administrations of pentobarbital. Results such as these indicate the need for information about the time characteristics of changes in biochemical systems induced by chemical agents if basic relations between chemical agents, biochemical events and behavior are to be understood.

5. Large "therapeutic index". The potential safety of new drugs is frequently expressed as a therapeutic index or ratio: LD_{50}/ED_{50}. The larger this value is, the greater is the margin of safety within which an agent may be used without danger of irreversible effects. In consideration of the criterion discussed in the preceding section, it is obviously desirable to seek agents with large therapeutic indexes.

A method of probit analysis for determining median behavioral doses, BD_{50}s, is described in Section IV D of the present report. This value represents the dose of a particular drug necessary to produce a significant change of performance in 50 per cent of the sample of subjects studied. For example, the BD_{50}s for motor and stress responses as measured during preliminary screening were found to be 100 mg/kg and 50 mg/kg, respectively, for oral administration of Librium; the BD_{50} for the straightaway escape response was 9 mg/kg. Comparing each of these with the LD_{50} of 2000 mg/kg provides therapeutic indexes of 20, 40 and 222. The differences between the ratios illustrates the wide dose range over which different behavior patterns may be sensitive to effects of the same drug. It emphasizes the importance in behavioral screening of chemical agents of not depending solely on a single or even upon several related measures of behavior; a variety of measures representing different aspects of behavior can provide a profile of effects which more usefully describes the behavioral toxicity of a drug and make possible comparisons with patterns for other agents.

6. Tolerance and sensitizing effects. Although circumstances under which incapacitating agents might be employed for military purposes make it desirable that such agents be effective with a single administration, it is possible that target personnel might be exposed to prolonged or repeated administrations of an agent. The effects of repeated exposure may vary with different agents from increasing tolerance to increasing sensitivity. In the present project Latin square research designs were used as the major approach to studying carry-over effects and for determining any influences which the order of doses may have had when a sequence of administrations involved more than one dose level.

Significant carry-over effects were found to occur with certain drugs, e.g., Librium, and not with others, e.g., pentobarbital. In the case of the present agents, the trend was in the direction of sensitization, i.e., of greater magnitudes in changes of performance from predrug baseline levels. No significant dose-order effects were found.

Carry-over effects from one drug administration to another may introduce serious complications of considerable practical importance in behavioral screening programs for incapacitating agents where the same animals are used for extended periods of time during which drug administrations include not only different doses of the same agent, but also agents with different chemomorphologies. It is difficult to see how data obtained under such conditions can be meaningfully interpreted as screening programs are often carried out. With adequate research designs, e.g., Latin square, it is possible to determine whether or not carry-over effects have occurred in order that they may be considered in interpreting results obtained. Had the project continued, other research designs, which might have assisted in the interpretation, would have been studied; some possibilities are mentioned in Section II of this report.

An obvious alternative approach is to eliminate such effects, if possible. One means of doing this is to allow adequate intervals between successive drug administrations. However, adequate intervals must be determined empirically for the experimental conditions under which final tests are to be carried out. Since the interval is defined by the duration of the slowest "recovery process" among the three major variables involved in the drug-biochemical system -

behavior interaction, it is necessary to know the time characteristics of each. It is not adequate to base inter-treatment intervals upon knowledge of the time-response relations for behavior changes only: in the present experiments, preliminary studies showed that decrements in level of performance following administration of Librium disappeared well within the seven-day interval between successive administrations, yet significant carry-over effects, presumably related to continued effects of the drug on some biochemical system(s), were found.

VII. REFERENCES

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